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Gene essentiality and synthetic lethality in haploid human cells

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Although the genes essential for life have been identified in less complex model organisms, their elucidation in human cells has been hindered by technical barriers. We use extensive mutagenesis in haploid human cells to identify approximately 2,000 genes required for optimal fitness under culture conditions. To study the principles of genetic interactions in human cells we created a synthetic lethality network focused on the secretory pathway based exclusively on mutations. This revealed a genetic crosstalk governing Golgi homeostasis, an additional subunit of the human oligosaccharyltransferase complex, and a Phosphatidylinositol 4-Kinase Beta adaptor hijacked by viruses. The synthetic lethality map parallels observations made in yeast and projects a route forward to reveal genetic networks in diverse aspects of human cell biology.

Single cell organisms can tolerate inactivating mutations in the majority of genes (*1–3*), but it is unclear whether human cells require more essential genes due to increased complexity, or fewer, due to added redundancy. To study this we used mutagenesis in the near-haploid chronic myeloid leukemia (CML) cell line KBM7 (karyotype 25, XY, +8, Ph+), and its non-hematopoietic derivative HAP1 which is haploid for all chromosomes (fig. S1A) (*4*). More than 34.3 million and 65.9 million gene-trap integrations were identified in KBM7 and HAP1 cells, respectively. The employed gene-trap vector was unidirectional by design (fig. S1B) and for most genes the number of intronic integrations in the sense direction was similar to that in the antisense direction (e.g., pro-apoptotic factor *BBC3*) (Fig. 1, A and B) (*5–7*). For a fraction of genes, however, disruptive mutations were underrepresented, indicative of impaired fitness: some genes (e.g., *STAT5B*) appeared essential in one cell type (fig. S1C), whereas others in both (such as *RPL13A)* (Fig. 1, A and B, and tables S1 to S3).

2,054 genes in KBM7 cells (table S1 and figs. S2 and S3) and 2,181 genes in HAP1 (table S2 and figs. S2 and S3) appeared to be needed for viability or optimal fitness under the experimental growth conditions (referred to as 'essential' although the approach does not distinguish between the two). The 1,734 genes identified in both cell lines were designated as 'core essentialome' (table S3). Importantly in KBM7 cells, genes on chromosome 8 (present in two copies) tolerated disruptive mutations, underscoring the specificity of the approach (fig. S1D). Furthermore nearly all subunits of the proteasome were identified as essential (fig. S4). In general, essential genes are overrepresented in categories such as translation or transcription but not signaling (Fig. 1C and figs. S5 and S6).

Many genes required for fitness in yeast were also essential in human cells. Exceptions were largely explained by paralogs in the human genome or by yeast-specific requirements (fig. S7A and table S4) (*1*). We estimated the evolutionary age of essential genes and found that 77% emerged in pre-metazoans ('*old*' essential genes) (fig. S7B). Essential genes had fewer paralogs and higher protein abundance and contained fewer single nucleotide polymorphisms (SNPs) predicted to impair function (Fig. 1D). Proteins encoded by essential genes displayed more protein-protein interactions (fig. S8, A to D) and these occurred more frequently with other essential proteins (49.8%) (fig. S9A) and within the same functional category (fig. S5B). Remarkably, the products of *new* essential genes are more often connected with *old* rather than other *new* essential gene products, suggesting that they largely function within ancient molecular machineries (fig. S9, B and C).

To identify proteins interacting with products of 18 uncharacterized essential genes we used tandem affinity purification coupled to mass spectrometry (fig. S10). Interactors were frequently essential proteins (52.4%, $P < 2.5E\text{-}36$, hypergeometric test) involved in processes like splicing, translation, and trafficking (fig. S11 and table S5). The small transmembrane protein TMEM258 associated with components of the conserved oligosaccharyltransferase (OST) com-

plex (Fig. 2A and fig. S12A) essential for protein N-glycosylation (*8*). TMEM258 localized to the endoplasmic reticulum (fig. S12B) and depletion (fig. S12, C and D) impaired OST catalytic activity as monitored by hypoglycosylation of prosaposin (Fig. 2B) (*9*). This also rationalizes the observed clustering of TMEM258 with OST complex subunits in a recent genetic screen (*10*). Thus, TMEM258 constitutes a subunit of the human OST complex and although homology searches (fig. S12E) do not identify a yeast ortholog, TMEM258 may relate to the similarly sized yeast transmembrane protein OST5 (*11*).

Whereas most genes appear nonessential, their function may be buffered by other genes such that only simultaneous disruption is lethal (*12*–*15*). The frequency of such synthetic lethal interactions between human genes is debated and challenging to address experimentally (*16*, *17*). We studied the small guanosine triphosphatases (GTPases) RAB1A and RAB1B, by creating individual knockout lines and assessing the genes needed for fitness in these backgrounds (Fig. 3A and fig. S13A). Whereas neither *RAB1A* nor *RAB1B* were essential in wild-type cells, *RAB1A* became indispensable in *RAB1B* knockout cells and vice versa (Fig. 3A and fig S13B). To explore the breadth of synthetic lethality we probed the secretory pathway using three independent knockout cell lines (fig. S14) for *RAB1A, RAB1B, GOSR1* (a subunit of the Golgi SNAP receptor), (*18*) and *TMEM165* (a Golgi-resident Ca^{2+}/H^{+} antiporter whose deficiency impairs glycosylation) (*19*) (Fig. 3B, figs. S15 and S16, and table S6). Most of their genetic interactions impinged on the secretory pathway (Fig. 3B and table S7) and many were found synthetic lethal with *PTAR1*. Synthetic lethality screens in PTAR1 deficient cells confirmed these genetic interactions and additionally identified the uncharacterized gene *C10orf76* (Fig. 3B and fig. S17A). Validation using *C10orf76* as query gene confirmed synthetic lethality with *PTAR1*, and (reciprocally) identified *TSSC1,* which was recently reported to interact with the Golgi associated retrograde protein complex (GARP) (Fig. 3B) (*20*). The human genes we studied display on average ~20 synthetic lethal interactions, a number comparable to that in yeast (*12*), although this varies between genes, with *PTAR1* (causing a fitness defect when deleted alone) having close to 60 interactions (fig. S17B). This illustrates that synthetic lethal interactions can be identified and validated using reciprocal haploid screens and that, similar to yeast, interactions frequently occur between genes whose products act in related processes (fig. S17B) (*13*, *16*). However, we acknowledge a caveat that this approach cannot readily distinguish between synthetic lethal or synthetic 'sick' interactions.

The impaired growth of *PTAR1*-deficient cells (table S2) was suppressed by loss of the Golgi factor GOLGA5 (*21*) (Fig. 4A and fig. S18). *PTAR1*-deficient cells had an abnormally dilated Golgi morphology (fig. S19A) which was partially corrected by co-deletion of *GOLGA5* (Fig. 4B and fig. S19B). Functionally, *PTAR1*-deficiency impaired glycosylation (fig. S19C) (*7*), possibly due to dysregulation of RAB proteins (*22*). Indeed *PTAR1*-deficient cells showed attenuated geranylgeranylation of RAB1A and RAB1B (fig. S19D). Partial correction of the Golgi morphology in cells lacking both *PTAR1* and *GOLGA5* could relate to the effect of GOLGA5, itself a RAB effector, on Golgi fragmentation (*21*, *23*). Thus, the interaction map reveals *PTAR1* and *GOLGA5* as opposing handles tuning Golgi morphology and homeostasis.

Genetic analysis suggested a link between the unstudied gene *C10orf76* and *PI4KB* which were both synthetic lethal with *PTAR1* (Fig. 3B). A host factor screen using coxsackievirus A10 also identified *C10orf76* as well as *PI4KB* (fig. S20, A and B) and a proteomics survey (*24*) suggested association between *C10orf76* and *PI4KB*. We confirmed this interaction in immunoprecipitation experiments with cells expressing FLAG-tagged C10orf76 (Fig. 4C). PI4KB regulates abundance of phosphatidylinositol 4-phosphate [PI(4)P] (*25*) and has a role in genome replication of various RNA viruses including coxsackieviruses (*26*). Infection studies confirmed that *C10orf76* knockout cells were particularly resistant to coxsackievirus A10 (fig. S20C). Although virus entry occurred normally, replication of viral RNA was decreased in *C10orf76* knockout cells (Fig. 4D and fig. S20D). Enteroviruses hijack PI4KB activity to construct 'replication factories' which were abundant in wild-type cells but rare in *C10orf76*-deficient cells (fig. S20E). Amounts of PI(4)P were decreased in these cells and Golgi retention of PI4KB after chemical inhibition (*27*) was largely dependent on C10orf76, which also localized to this compartment under these conditions (fig. S21, A and B). Thus, C10orf76 is a PI4KB-associated factor hijacked by specific picornaviruses for replication.

This study identifies approximately 2,000 genes required for optimal fitness of cultured haploid human cells. Despite technical limitations, the identification of gene essentiality shows high concordance with the gene-trap and CRISPR data reported in the accompanying manuscript of Wang *et al.* (supplementary text and fig. S22). This suggests that the increase in total number of genes in humans as compared to that in yeast yielded a system of higher complexity rather than more robustness through added redundancy. Nonessential human genes appear to frequently engage in synthetic lethal interactions. Our studies start to reveal an interconnected module of genetic interactions affecting the secretory pathway and link it to uncharacterized genes. The experimental strategy is applicable to various cellular processes and may help unravel the genetic network encoding a human cell.

REFERENCES AND NOTES

1. G. Giaever, A. M. Chu, L. Ni, C. Connelly, L. Riles, S. Véronneau, S. Dow, A. Lucau-Danila, K. Anderson, B. André, A. P. Arkin, A. Astromoff, M. El-Bakkoury, R. Bangham, R. Benito, S. Brachat, S. Campanaro, M. Curtiss, K. Davis, A. Deutschbauer, K. D. Entian, P. Flaherty, F. Foury, D. J. Garfinkel, M. Gerstein, D. Gotte, U. Güldener, J. H. Hegemann, S. Hempel, Z. Herman, D. F. Jaramillo, D. E. Kelly, S. L. Kelly, P. Kötter, D. LaBonte, D. C. Lamb, N. Lan, H. Liang, H. Liao, L. Liu, C. Luo, M. Lussier, R. Mao, P. Menard, S. L. Ooi, J. L. Revuelta, C. J. Roberts,

M. Rose, P. Ross-Macdonald, B. Scherens, G. Schimmack, B. Shafer, D. D. Shoemaker, S. Sookhai-Mahadeo, R. K. Storms, J. N. Strathern, G. Valle, M. Voet, G. Volckaert, C. Y. Wang, T. R. Ward, J. Wilhelmy, E. A. Winzeler, Y. Yang, G. Yen, E. Youngman, K. Yu, H. Bussey, J. D. Boeke, M. Snyder, P. Philippsen, R. W. Davis, M. Johnston, Functional profiling of the Saccharomyces cerevisiae genome. *Nature* **418**, 387–391 (2002). Medline doi:10.1038/nature00935

2. A. H. Tong, M. Evangelista, A. B. Parsons, H. Xu, G. D. Bader, N. Pagé, M. Robinson, S. Raghibizadeh, C. W. Hogue, H. Bussey, B. Andrews, M. Tyers, C. Boone, Systematic genetic analysis with ordered arrays of yeast deletion mutants. *Science* **294**, 2364–2368 (2001). Medline doi:10.1126/science.1065810
3. S. Mnaimneh, A. P. Davierwala, J. Haynes, J. Moffat, W. T. Peng, W. Zhang, X. Yang, J. Pootoolal, G. Chua, A. Lopez, M. Trochesset, D. Morse, N. J. Krogan, S. L. Hiley, Z. Li, Q. Morris, J. Grigull, N. Mitsakakis, C. J. Roberts, J. F. Greenblatt, C. Boone, C. A. Kaiser, B. J. Andrews, T. R. Hughes, Exploration of essential gene functions via titratable promoter alleles. *Cell* **118**, 31–44 (2004). Medline doi:10.1016/j.cell.2004.06.013
4. J. E. Carette, M. Raaben, A. C. Wong, A. S. Herbert, G. Obernosterer, N. Mulherkar, A. I. Kuehne, P. J. Kranzusch, A. M. Griffin, G. Ruthel, P. Dal Cin, J. M. Dye, S. P. Whelan, K. Chandran, T. R. Brummelkamp, Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. *Nature* **477**, 340–343 (2011). Medline doi:10.1038/nature10348
5. J. E. Carette, C. P. Guimaraes, I. Wuethrich, V. A. Blomen, M. Varadarajan, C. Sun, G. Bell, B. Yuan, M. K. Muellner, S. M. Nijman, H. L. Ploegh, T. R. Brummelkamp, Global gene disruption in human cells to assign genes to phenotypes by deep sequencing. *Nat. Biotechnol.* **29**, 542–546 (2011). Medline doi:10.1038/nbt.1857
6. J. E. Carette, C. P. Guimaraes, M. Varadarajan, A. S. Park, I. Wuethrich, A. Godarova, M. Kotecki, B. H. Cochran, E. Spooner, H. L. Ploegh, T. R. Brummelkamp, Haploid genetic screens in human cells identify host factors used by pathogens. *Science* **326**, 1231–1235 (2009). Medline
7. L. T. Jae, M. Raaben, M. Riemersma, E. van Beusekom, V. A. Blomen, A. Velds, R. M. Kerkhoven, J. E. Carette, H. Topaloglu, P. Meinecke, M. W. Wessels, D. J. Lefeber, S. P. Whelan, H. van Bokhoven, T. R. Brummelkamp, Deciphering the glycosylome of dystroglycanopathies using haploid screens for lassa virus entry. *Science* **340**, 479–483 (2013). Medline doi:10.1126/science.1233675
8. D. J. Kelleher, R. Gilmore, An evolving view of the eukaryotic oligosaccharyltransferase. *Glycobiology* **16**, 47R–62R (2006). Medline doi:10.1093/glycob/cwj066
9. A. Dumax-Vorzet, P. Roboti, S. High, OST4 is a subunit of the mammalian oligosaccharyltransferase required for efficient N-glycosylation. *J. Cell Sci.* **126**, 2595–2606 (2013). Medline doi:10.1242/jcs.115410
10. O. Parnas, M. Jovanovic, T. M. Eisenhaure, R. H. Herbst, A. Dixit, C. J. Ye, D. Przybylski, R. J. Platt, I. Tirosh, N. E. Sanjana, O. Shalem, R. Satija, R. Raychowdhury, P. Mertins, S. A. Carr, F. Zhang, N. Hacohen, A. Regev, A Genome-wide CRISPR Screen in Primary Immune Cells to Dissect Regulatory Networks. *Cell* **162**, 675–686 (2015). Medline doi:10.1016/j.cell.2015.06.059
11. G. Reiss, S. te Heesen, R. Gilmore, R. Zufferey, M. Aebi, A specific screen for oligosaccharyltransferase mutations identifies the 9 kDa OST5 protein required for optimal activity in vivo and in vitro. *EMBO J.* **16**, 1164–1172 (1997). Medline doi:10.1093/emboj/16.6.1164
12. A. H. Tong, G. Lesage, G. D. Bader, H. Ding, H. Xu, X. Xin, J. Young, G. F. Berriz, R. L. Brost, M. Chang, Y. Chen, X. Cheng, G. Chua, H. Friesen, D. S. Goldberg, J. Haynes, C. Humphries, G. He, S. Hussein, L. Ke, N. Krogan, Z. Li, J. N. Levinson, H. Lu, P. Ménard, C. Munyana, A. B. Parsons, O. Ryan, R. Tonikian, T. Roberts, A. M. Sdicu, J. Shapiro, B. Sheikh, B. Suter, S. L. Wong, L. V. Zhang, H. Zhu, C. G. Burd, S. Munro, C. Sander, J. Rine, J. Greenblatt, M. Peter, A. Bretscher, G. Bell, F. P. Roth, G. W. Brown, B. Andrews, H. Bussey, C. Boone, Global mapping of the yeast genetic interaction network. *Science* **303**, 808–813 (2004). Medline doi:10.1126/science.1091317
13. J. L. Hartman 4th, B. Garvik, L. Hartwell, Principles for the buffering of genetic variation. *Science* **291**, 1001–1004 (2001). Medline doi:10.1126/science.291.5506.1001
14. C. Boone, H. Bussey, B. J. Andrews, Exploring genetic interactions and networks with yeast. *Nat. Rev. Genet.* **8**, 437–449 (2007). Medline doi:10.1038/nrg2085
15. M. Schuldiner, S. R. Collins, N. J. Thompson, V. Denic, A. Bhamidipati, T. Punna, J. Ihmels, B. Andrews, C. Boone, J. F. Greenblatt, J. S. Weissman, N. J. Krogan, Exploration of the function and organization of the yeast early secretory pathway through an epistatic miniarray profile. *Cell* **123**, 507–519 (2005). Medline doi:10.1016/j.cell.2005.08.031
16. S. M. Nijman, S. H. Friend, Cancer. Potential of the synthetic lethality principle. *Science* **342**, 809–811 (2013). Medline doi:10.1126/science.1244669
17. J. Tischler, B. Lehner, A. G. Fraser, Evolutionary plasticity of genetic interaction networks. *Nat. Genet.* **40**, 390–391 (2008). Medline doi:10.1038/ng.114
18. V. N. Subramaniam, F. Peter, R. Philp, S. H. Wong, W. Hong, GS28, a 28-kilodalton Golgi SNARE that participates in ER-Golgi transport. *Science* **272**, 1161–1163 (1996). Medline doi:10.1126/science.272.5265.1161
19. D. Demaegd, F. Foulquier, A. S. Colinet, L. Gremillon, D. Legrand, P. Mariot, E. Peiter, E. Van Schaftingen, G. Matthijs, P. Morsomme, Newly characterized Golgi-localized family of proteins is involved in calcium and pH homeostasis in yeast and human cells. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 6859–6864 (2013). Medline doi:10.1073/pnas.1219871110
20. E. L. Huttlin, L. Ting, R. J. Bruckner, F. Gebreab, M. P. Gygi, J. Szpyt, S. Tam, G. Zarraga, G. Colby, K. Baltier, R. Dong, V. Guarani, L. P. Vaites, A. Ordureau, R. Rad, B. K. Erickson, M. Wühr, J. Chick, B. Zhai, D. Kolippakkam, J. Mintseris, R. A. Obar, T. Harris, S. Artavanis-Tsakonas, M. E. Sowa, P. De Camilli, J. A. Paulo, J. W. Harper, S. P. Gygi, The BioPlex Network: A Systematic Exploration of the Human Interactome. *Cell* **162**, 425–440 (2015). Medline doi:10.1016/j.cell.2015.06.043
21. A. Diao, D. Rahman, D. J. Pappin, J. Lucocq, M. Lowe, The coiled-coil membrane protein golgin-84 is a novel rab effector required for Golgi ribbon formation. *J. Cell Biol.* **160**, 201–212 (2003). Medline doi:10.1083/jcb.200207045
22. W. L. Charng, S. Yamamoto, M. Jaiswal, V. Bayat, B. Xiong, K. Zhang, H. Sandoval, G. David, S. Gibbs, H. C. Lu, K. Chen, N. Giagtzoglou, H. J. Bellen, Drosophila Tempura, a novel protein prenyltransferase α subunit, regulates notch signaling via Rab1 and Rab11. *PLOS Biol.* **12**, e1001777 (2014). Medline doi:10.1371/journal.pbio.1001777
23. A. Rejman Lipinski, J. Heymann, C. Meissner, A. Karlas, V. Brinkmann, T. F. Meyer, D. Heuer, Rab6 and Rab11 regulate Chlamydia trachomatis development and golgin-84-dependent Golgi fragmentation. *PLOS Pathog.* **5**, e1000615 (2009). Medline doi:10.1371/journal.ppat.1000615
24. A. L. Greninger, G. M. Knudsen, M. Betegon, A. L. Burlingame, J. L. DeRisi, ACBD3 interaction with TBC1 domain 22 protein is differentially affected by enteroviral and kobuviral 3A protein binding. *MBio* **4**, e00098–e00013 (2013). Medline doi:10.1128/mBio.00098-13
25. T. R. Graham, C. G. Burd, Coordination of Golgi functions by phosphatidylinositol 4-kinases. *Trends Cell Biol.* **21**, 113–121 (2011). Medline doi:10.1016/j.tcb.2010.10.002
26. N. Y. Hsu, O. Ilnytska, G. Belov, M. Santiana, Y. H. Chen, P. M. Takvorian, C. Pau, H. van der Schaar, N. Kaushik-Basu, T. Balla, C. E. Cameron, E. Ehrenfeld, F. J. van Kuppeveld, N. Altan-Bonnet, Viral reorganization of the secretory pathway generates distinct organelles for RNA replication. *Cell* **141**, 799–811 (2010). Medline doi:10.1016/j.cell.2010.03.050
27. H. M. van der Schaar, L. van der Linden, K. H. Lanke, J. R. Strating, G. Pürstinger, E. de Vries, C. A. de Haan, J. Neyts, F. J. van Kuppeveld, Coxsackievirus mutants that can bypass host factor PI4KIIIβ and the need for high levels of PI4P lipids for replication. *Cell Res.* **22**, 1576–1592 (2012). Medline doi:10.1038/cr.2012.129
28. J. Seebacher, A. C. Gavin, SnapShot: Protein-protein interaction networks. *Cell* **144**, 1000–1000.e1, 1000.e1 (2011). Medline doi:10.1016/j.cell.2011.02.025
29. M. Newmann, *Networks: An Introduction*. (Oxford University Press, ed. 1 edition 2010), pp. 720.
30. C. M. Toledo, J. A. Herman, J. B. Olsen, Y. Ding, P. Corrin, E. J. Girard, J. M. Olson, A. Emili, J. G. DeLuca, P. J. Paddison, BuGZ is required for Bub3 stability, Bub1 kinetochore function, and chromosome alignment. *Dev. Cell* **28**, 282–294 (2014). Medline doi:10.1016/j.devcel.2013.12.014
31. H. Jiang, X. He, S. Wang, J. Jia, Y. Wan, Y. Wang, R. Zeng, J. Yates 3rd, X. Zhu, Y. Zheng, A microtubule-associated zinc finger protein, BuGZ, regulates mitotic chromosome alignment by ensuring Bub3 stability and kinetochore targeting. *Dev. Cell* **28**, 268–281 (2014). Medline doi:10.1016/j.devcel.2013.12.013
32. T. Blondal, A. Thorisdottir, U. Unnsteinsdottir, S. Hjorleifsdottir, A. Aevarsson, S. Ernstsson, O. H. Fridjonsson, S. Skirnisdottir, J. O. Wheat, A. G. Hermannsdottir, S. T. Sigurdsson, G. O. Hreggvidsson, A. V. Smith, J. K. Kristjansson, Isolation and characterization of a thermostable RNA ligase 1 from a Thermus scotoductus bacteriophage TS2126 with good single-stranded DNA ligation properties. *Nucleic Acids Res.* **33**, 135–142 (2005). Medline doi:10.1093/nar/gki149
33. B. Langmead, C. Trapnell, M. Pop, S. L. Salzberg, Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* **10**, R25

(2009). Medline doi:10.1186/gb-2009-10-3-r25
34. J. Harrow, A. Frankish, J. M. Gonzalez, E. Tapanari, M. Diekhans, F. Kokocinski, B. L. Aken, D. Barrell, A. Zadissa, S. Searle, I. Barnes, A. Bignell, V. Boychenko, T. Hunt, M. Kay, G. Mukherjee, J. Rajan, G. Despacio-Reyes, G. Saunders, C. Steward, R. Harte, M. Lin, C. Howald, A. Tanzer, T. Derrien, J. Chrast, N. Walters, S. Balasubramanian, B. Pei, M. Tress, J. M. Rodriguez, I. Ezkurdia, J. van Baren, M. Brent, D. Haussler, M. Kellis, A. Valencia, A. Reymond, M. Gerstein, R. Guigó, T. J. Hubbard, GENCODE: The reference human genome annotation for The ENCODE Project. *Genome Res.* **22**, 1760–1774 (2012). Medline doi:10.1101/gr.135350.111
35. F. P. Breitwieser, A. Müller, L. Dayon, T. Köcher, A. Hainard, P. Pichler, U. Schmidt-Erfurth, G. Superti-Furga, J. C. Sanchez, K. Mechtler, K. L. Bennett, J. Colinge, General statistical modeling of data from protein relative expression isobaric tags. *J. Proteome Res.* **10**, 2758–2766 (2011). Medline doi:10.1021/pr1012784
36. Z. Dosztányi, V. Csizmok, P. Tompa, I. Simon, IUPred: Web server for the prediction of intrinsically unstructured regions of proteins based on estimated energy content. *Bioinformatics* **21**, 3433–3434 (2005). Medline doi:10.1093/bioinformatics/bti541
37. T. Bürckstümmer, C. Banning, P. Hainzl, R. Schobesberger, C. Kerzendorfer, F. M. Pauler, D. Chen, N. Them, F. Schischlik, M. Rebsamen, M. Smida, F. Fece de la Cruz, A. Lapao, M. Liszt, B. Eizinger, P. M. Guenzl, V. A. Blomen, T. Konopka, B. Gapp, K. Parapatics, B. Maier, J. Stöckl, W. Fischl, S. Salic, M. R. Taba Casari, S. Knapp, K. L. Bennett, C. Bock, J. Colinge, R. Kralovics, G. Ammerer, G. Casari, T. R. Brummelkamp, G. Superti-Furga, S. M. Nijman, A reversible gene trap collection empowers haploid genetics in human cells. *Nat. Methods* **10**, 965–971 (2013). Medline doi:10.1038/nmeth.2609
38. W. Huang, B. T. Sherman, R. A. Lempicki, Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* **4**, 44–57 (2009). Medline doi:10.1038/nprot.2008.211
39. W. Huang, B. T. Sherman, R. A. Lempicki, Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res.* **37**, 1–13 (2009). Medline doi:10.1093/nar/gkn923
40. H. Hermjakob, L. Montecchi-Palazzi, C. Lewington, S. Mudali, S. Kerrien, S. Orchard, M. Vingron, B. Roechert, P. Roepstorff, A. Valencia, H. Margalit, J. Armstrong, A. Bairoch, G. Cesareni, D. Sherman, R. Apweiler, IntAct: An open source molecular interaction database. *Nucleic Acids Res.* **32**, D452–D455 (2004). Medline doi:10.1093/nar/gkh052
41. C. Stark, B. J. Breitkreutz, T. Reguly, L. Boucher, A. Breitkreutz, M. Tyers, BioGRID: A general repository for interaction datasets. *Nucleic Acids Res.* **34**, D535–D539 (2006). Medline doi:10.1093/nar/gkj109
42. T. S. Keshava Prasad, R. Goel, K. Kandasamy, S. Keerthikumar, S. Kumar, S. Mathivanan, D. Telikicherla, R. Raju, B. Shafreen, A. Venugopal, L. Balakrishnan, A. Marimuthu, S. Banerjee, D. S. Somanathan, A. Sebastian, S. Rani, S. Ray, C. J. Harrys Kishore, S. Kanth, M. Ahmed, M. K. Kashyap, R. Mohmood, Y. L. Ramachandra, V. Krishna, B. A. Rahiman, S. Mohan, P. Ranganathan, S. Ramabadran, R. Chaerkady, A. Pandey, Human Protein Reference Database—2009 update. *Nucleic Acids Res.* **37** (Database), D767–D772 (2009). Medline doi:10.1093/nar/gkn892
43. I. Xenarios, D. W. Rice, L. Salwinski, M. K. Baron, E. M. Marcotte, D. Eisenberg, DIP: The database of interacting proteins. *Nucleic Acids Res.* **28**, 289–291 (2000). Medline doi:10.1093/nar/28.1.289
44. A. Chatr-aryamontri, A. Ceol, L. M. Palazzi, G. Nardelli, M. V. Schneider, L. Castagnoli, G. Cesareni, MINT: The Molecular INTeraction database. *Nucleic Acids Res.* **35** (Database), D572–D574 (2007). Medline doi:10.1093/nar/gkl950
45. D. J. Lynn, G. L. Winsor, C. Chan, N. Richard, M. R. Laird, A. Barsky, J. L. Gardy, F. M. Roche, T. H. Chan, N. Shah, R. Lo, M. Naseer, J. Que, M. Yau, M. Acab, D. Tulpan, M. D. Whiteside, A. Chikatamarla, B. Mah, T. Munzner, K. Hokamp, R. E. Hancock, F. S. Brinkman, InnateDB: Facilitating systems-level analyses of the mammalian innate immune response. *Mol. Syst. Biol.* **4**, 218 (2008). Medline doi:10.1038/msb.2008.55
46. G. Launay, R. Salza, D. Multedo, N. Thierry-Mieg, S. Ricard-Blum, MatrixDB, the extracellular matrix interaction database: Updated content, a new navigator and expanded functionalities. *Nucleic Acids Res.* **43** (D1), D321–D327 (2015). Medline doi:10.1093/nar/gku1091
47. A. Ruepp, B. Waegele, M. Lechner, B. Brauner, I. Dunger-Kaltenbach, G. Fobo, G. Frishman, C. Montrone, H. W. Mewes, CORUM: The comprehensive resource of mammalian protein complexes—2009. *Nucleic Acids Res.* **38** (Database), D497–D501 (2010). Medline doi:10.1093/nar/gkp914
48. C. F. Schaefer, K. Anthony, S. Krupa, J. Buchoff, M. Day, T. Hannay, K. H. Buetow, PID: The Pathway Interaction Database. *Nucleic Acids Res.* **37** (Database), D674–D679 (2009). Medline doi:10.1093/nar/gkn653
49. S. Powell, D. Szklarczyk, K. Trachana, A. Roth, M. Kuhn, J. Muller, R. Arnold, T. Rattei, I. Letunic, T. Doerks, L. J. Jensen, C. von Mering, P. Bork, eggNOG v3.0: Orthologous groups covering 1133 organisms at 41 different taxonomic ranges. *Nucleic Acids Res.* **40** (D1), D284–D289 (2012). Medline doi:10.1093/nar/gkr1060
50. T. Glatter, A. Wepf, R. Aebersold, M. Gstaiger, An integrated workflow for charting the human interaction proteome: Insights into the PP2A system. *Mol. Syst. Biol.* **5**, 237 (2009). Medline doi:10.1038/msb.2008.75
51. L. T. Jae, M. Raaben, A. S. Herbert, A. I. Kuehne, A. S. Wirchnianski, T. K. Soh, S. H. Stubbs, H. Janssen, M. Damme, P. Saftig, S. P. Whelan, J. M. Dye, T. R. Brummelkamp, Lassa virus entry requires a trigger-induced receptor switch. *Science* **344**, 1506–1510 (2014). Medline doi:10.1126/science.1252480
52. E. Campeau, V. E. Ruhl, F. Rodier, C. L. Smith, B. L. Rahmberg, J. O. Fuss, J. Campisi, P. Yaswen, P. K. Cooper, P. D. Kaufman, A versatile viral system for expression and depletion of proteins in mammalian cells. *PLOS ONE* **4**, e6529 (2009). Medline
53. E. L. Rudashevskaya, R. Sacco, K. Kratochwill, M. L. Huber, M. Gstaiger, G. Superti-Furga, K. L. Bennett, A method to resolve the composition of heterogeneous affinity-purified protein complexes assembled around a common protein by chemical cross-linking, gel electrophoresis and mass spectrometry. *Nat. Protoc.* **8**, 75–97 (2013). Medline doi:10.1038/nprot.2012.133
54. M. Varjosalo, R. Sacco, A. Stukalov, A. van Drogen, M. Planyavsky, S. Hauri, R. Aebersold, K. L. Bennett, J. Colinge, M. Gstaiger, G. Superti-Furga, Interlaboratory reproducibility of large-scale human protein-complex analysis by standardized AP-MS. *Nat. Methods* **10**, 307–314 (2013). Medline doi:10.1038/nmeth.2400
55. M. L. Huber, R. Sacco, K. Parapatics, A. Skucha, K. Khamina, A. C. Müller, E. L. Rudashevskaya, K. L. Bennett, abFASP-MS: Affinity-based filter-aided sample preparation mass spectrometry for quantitative analysis of chemically labeled protein complexes. *J. Proteome Res.* **13**, 1147–1155 (2014). Medline doi:10.1021/pr4009892
56. D. N. Perkins, D. J. Pappin, D. M. Creasy, J. S. Cottrell, Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis* **20**, 3551–3567 (1999). Medline doi:10.1002/(SICI)1522-2683(19991201)20:18<3551::AID-ELPS3551>3.0.CO;2-2
57. J. Colinge, A. Masselot, M. Giron, T. Dessingy, J. Magnin, OLAV: Towards high-throughput tandem mass spectrometry data identification. *Proteomics* **3**, 1454–1463 (2003). Medline doi:10.1002/pmic.200300485
58. A. Pichlmair, K. Kandasamy, G. Alvisi, O. Mulhern, R. Sacco, M. Habjan, M. Binder, A. Stefanovic, C. A. Eberle, A. Goncalves, T. Bürckstümmer, A. C. Müller, A. Fauster, C. Holze, K. Lindsten, S. Goodbourn, G. Kochs, F. Weber, R. Bartenschlager, A. G. Bowie, K. L. Bennett, J. Colinge, G. Superti-Furga, Viral immune modulators perturb the human molecular network by common and unique strategies. *Nature* **487**, 486–490 (2012). Medline doi:10.1038/nature11289
59. M. Lavallée-Adam, P. Cloutier, B. Coulombe, M. Blanchette, Modeling contaminants in AP-MS/MS experiments. *J. Proteome Res.* **10**, 886–895 (2011). Medline doi:10.1021/pr100795z
60. L. Cong, F. A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P. D. Hsu, X. Wu, W. Jiang, L. A. Marraffini, F. Zhang, Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819–823 (2013). Medline doi:10.1126/science.1231143
61. T. O. Auer, K. Duroure, A. De Cian, J. P. Concordet, F. Del Bene, Highly efficient CRISPR/Cas9-mediated knock-in in zebrafish by homology-independent DNA repair. *Genome Res.* **24**, 142–153 (2014). Medline doi:10.1101/gr.161638.113

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SUPPLEMENTARY MATERIALS
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Materials and Methods
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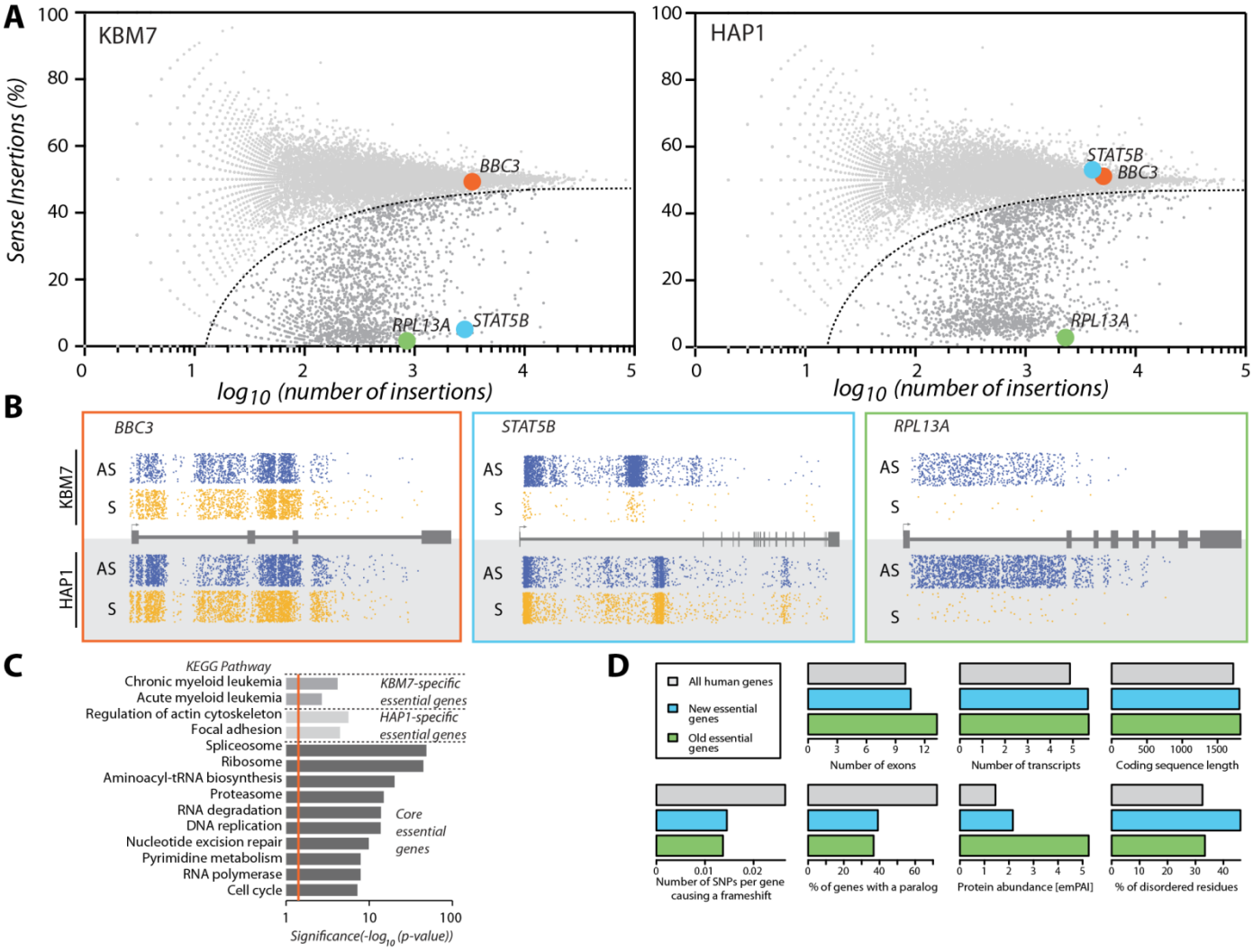

Fig. 1. Identification of genes required for fitness in KBM7 and HAP1 cells through insertional mutagenesis. (**A**) Unique gene-trap insertions were mapped in KBM7 and HAP1 cells, and their orientation relative to the affected genes was counted. Per gene the percentage of sense orientation gene-trap insertions (Y-axis) and the total number of insertions in a particular gene (X-axis) are plotted. (**B**) Gene-trap insertions identified in the sense (S, yellow) or antisense orientation (AS, blue) in a non-essential gene (*BBC3*), a gene essential only in KBM7 cells (*STAT5B*), and a gene essential in both cell lines (*RPL13A*). (**C**) KEGG pathway enrichment analysis of essential genes shared between or unique to KBM7 or HAP1 cells. (**D**) Properties of 'new' and 'old' essential genes compared to the human genome. Averages for the sets are displayed, except for protein abundance where median emPAI values are shown.

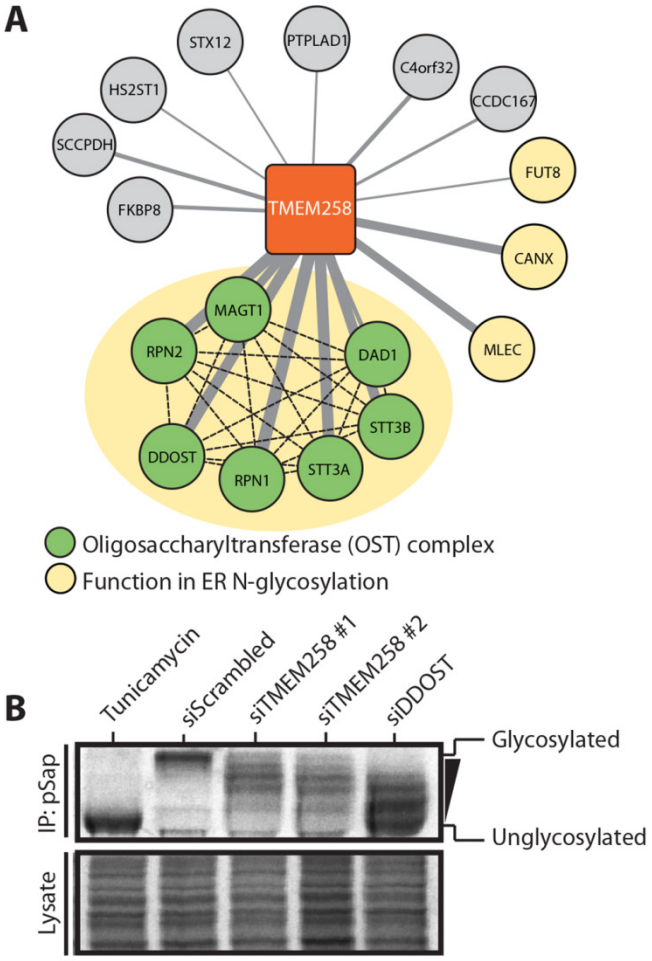

Fig. 2. The essential gene *TMEM258* encodes a component of the OST complex. (**A**) High-confidence protein-protein interactions associated with TMEM258. Green proteins indicate members of the oligosaccharyltransferase (OST) complex. Dashed lines indicate the OST complex subnetwork. (**B**) Effects of depletion of TMEM258 with siRNAs on the glycosylation of endogenous prosaposin. Cells were pulsed with ^{35}S-methionine/cysteine, lysed and subjected to immunoprecipitation using anti-prosaposin antibodies. Precipitated proteins were detected by phosphorimaging and hypoglycosylated prosaposin species are indicated. Tunicamycin treatment and depletion of the established OST subunit DDOST served as positive controls.

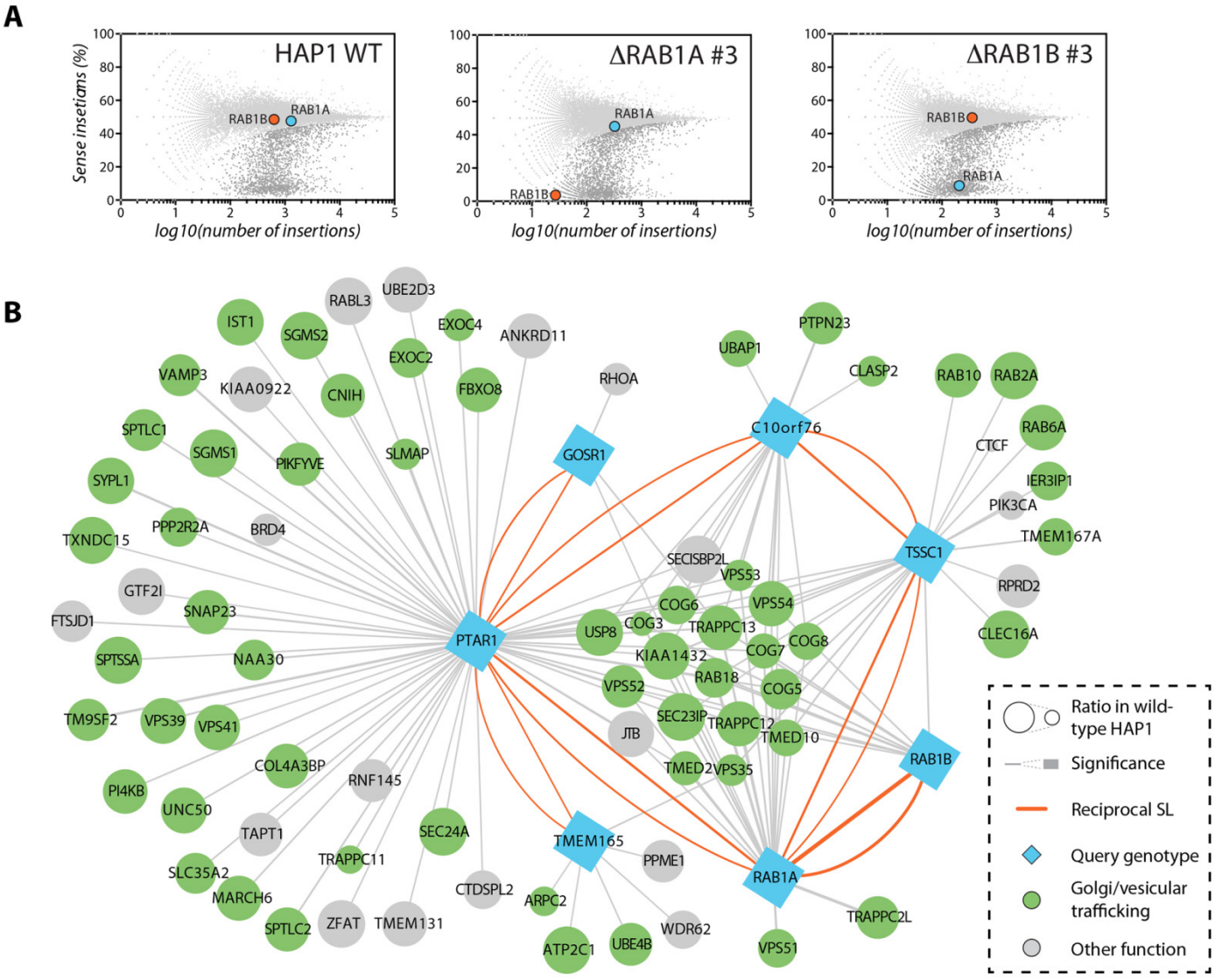

Fig. 3. Synthetic lethality network generated based on mutations. (**A**) Essentiality of *RAB1A* and *RAB1B* in wild-type HAP1 cells and cells deficient for *RAB1A* or *RAB1B*. (**B**) Genetic interaction network indicating synthetic lethal/sick interactions that were identified by scoring genes for fitness reduction in three nuclease-generated knockout clones per genotype. This revealed an interconnected network with many genes that could be functionally assigned to the secretory pathway (labeled in green). Reciprocal interactions, scored in either query genotype, are indicated by red edges. Edge thickness reflects the effect size of the interaction (compared to wild-type cells).

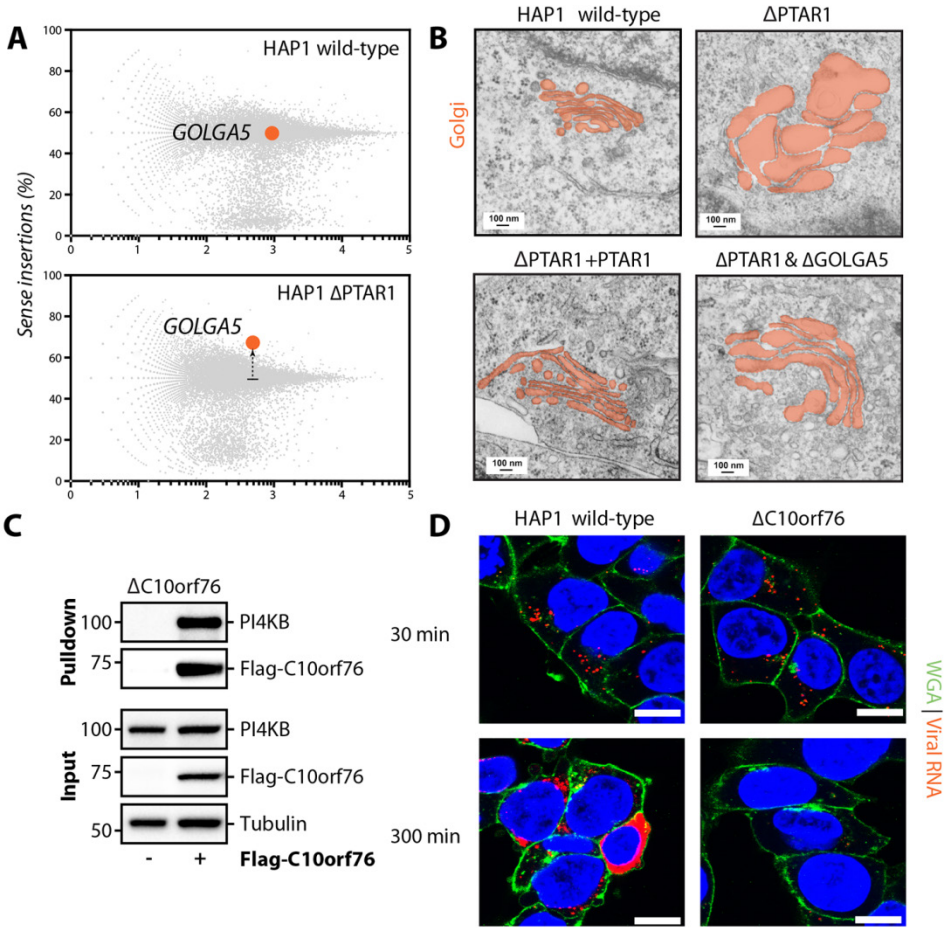

Fig. 4. Roles of *PTAR1* and *C10orf76* in Golgi homeostasis and virus replication. (**A**) A bias for sense-orientation integrations in *GOLGA5* observed in *PTAR1*-deficient HAP1 cells but not wild-type cells. (**B**) Electron micrographs of the Golgi apparatus (orange highlight) in the indicated genotypes. (**C**) Interaction of Flag-tagged C10orf76 with PI4KB in HAP1 cells detected by immunoprecipitation using anti-Flag antibodies. (**D**) Coxsackievirus A10 amplification in wild-type and *C10orf76*-deficient cells measured by single molecule fluorescent in situ hybridization (smFISH) to localize individual viral genomes (red). Intracellular viral RNA was first detected after 30 min. Increased RNA signal after 300 min indicates RNA replication.