**SUPPLEMENTAL FIGURE LEGENDS**

**Figure S1: Summary of shRNA production for human disease orthologs.** Production status of shRNA for human disease gene ortholog reagent libraries, organized by top-level medical subject heading (MeSH) terms. HuDis-3000 is the full set of ‘HuDis’ gene set orthologs with DIOPT vs.3 scores >=2 (best score, forward or reverse search) or DIOPT vs.3 score >=4. HuDis-670 is a smaller set of high confidence orthologs with DIOPT vs.3 scores >=8.

**Figure S2: Summary of sgRNA production for human disease orthologs.** Production status of TRiP-KO and TRiP-OE sgRNA for human disease gene ortholog reagent libraries, organized by top-level medical subject heading (MeSH) terms.

**SUPPLEMENTAL METHODS**

**The TRiP RNAi collection**

**RNAi vectors** The TRiP has generated a series of 22 knockdown vectors, the VALIUM series vectors (for Vermilion-AttB-Loxp-Intron-UAS-MCS), to facilitate incorporation of RNAi hairpinsinto attP landing sites (Ni *et al.* 2008; Ni *et al.* 2009; Ni *et al.* 2011; Perkins *et al.* 2015). All VALIUM vectors contain a wild type copy of *vermilion* as a selectable marker and an attB sequence to allow for phiC31 targeted integration at genomic attP landing sites (Groth *et al.* 2004). The VALIUM vectors were also designed with two pentamers of UAS sequences, one of which can be removed using the Cre/loxP system. In addition to manipulating the number of UAS sequences, the level of RNAi knockdown can also be altered by using Gal4 lines of various strengths, rearing flies at different temperatures, or via co-expression of UAS-Dicer2 (Dietzl *et al.* 2007) in the case of long double stranded RNAs (dsRNAs). The first-generation knockdown vectors chosen by the TRiP for RNAi stock production were VALIUM1 and VALIUM10. Both allow expression of long dsRNA hairpins, usually between 400 and 600 bp. These are very effective for RNAi in somatic tissues but are not as effective in the female germline (Ni et al., 2008; Ni et al., 2009). Subsequently, we showed that shRNAs containing a 21 bp targeting sequence embedded into a micro-RNA (miR-1) backbone are very effective for gene knockdown in both the germline and soma. For shRNA expression we developed the second-generation knockdown vectors, VALIUM20, VALIUM21 and VALIUM22 (Ni *et al.* 2011). Subsequent TRiP lines were generated with shRNAs in VALIUM20 (for knockdown in germline or soma) or VALIUM22 (mostly germline). Finally, since some researchers prefer to use *mini-white* as the selectable marker for transgenesis, we also generated new versions of the VALIUM vectors in which *vermilion* is replaced with *white* (WALIUM10, WALIUM20 and WALIUM22). Further information about the TRiP vectors in available at the TRiP website (<https://fgr.hms.harvard.edu/trip-plasmid-vector-sets>).The vectors are distributed by the [Drosophila Genome Resource Center](https://dgrc.bio.indiana.edu/vectors/Catalog) (<https://dgrc.bio.indiana.edu/>).

**Generation of the collection:** Our pipeline is as follows: (1) design shRNAs targeting each gene, (2) order oligos corresponding to shRNA sequences, (3) clone them into the expression vector and sequence verify, (4) inject into embryos, (5) isolate and balance transgenic flies, (6) sequence verify the transgenic stocks, (7) add stock information to our in-house database and coordinate with Flybase, (8) ship to BDSC for distribution.

To design an shRNA for a given gene, we first determine the sequences of all exons (or when appropriate, the sequences of exons common to all transcripts), then determine all possible 21 bp sub-sequences of the sense or antisense strands, and finally, compare these to all genes. Sub-sequences with 16 bp or longer stretches of identical nucleotide matches to other genes are removed from consideration. Each subsequence is assigned a score based on a formula by Vert et al. (Vert *et al.* 2006) and the top scoring sub-sequences are selected.

All fly stocks generated at the TRiP are inserted into one of two attP sites, attP40 on the left arm of the second chromosome at 25C6 or attP2 on the left arm of the third chromosome at 68A4. These sites were selected for their abilities to provide high levels of induced expression of the transgenes but maintain low basal expression when the transgenes are not induced (Markstein *et al.* 2008). The landing site chosen by the TRiP for hairpin insertion is guided first by the preference of the community member nominating the gene and second by the TRiP. If a TRiP stock for a particular gene is available in one location, a second TRiP stock for the same gene will be generated in the other location. shRNA constructs are generated individually in 96-well plates, or selected from shRNA libraries generated in VALIUM20 and VALIUM22 starting from a pool of 83,256 unique shRNA oligonucleotides synthesized on glass slide microarrays (Ni *et al.* 2011). Early in the project, constructs were injected individually into attP40- or attP2-bearing lines and transformants were recovered. As only one attB insert can integrate by phiC31-mediated recombination into an attP site (Groth *et al.* 2004), we later injected pools of constructs, established transgenic lines, and then subsequently characterized the inserted DNA by Sanger sequencing.

We produce the lines in the Perrimon lab, with injection companies such as Bestgene, or with the help of two outside groups, the National Institute of Genetics (NIG) in Japan (coordinated by Drs. Shu Kondo and Ryu Ueda) and the THFC at Tsinghua University in China (coordinated by Dr. Jianquan Ni). Importantly, these outside labs use established TRiP nomenclature and send the lines they generate to the TRiP at HMS, where they are checked for quality. All completed stocks are annotated on the TRiP website (http://fgr.hms.harvard.edu/) and on FlyBase, then transferred as soon as possible to the BDSC for distribution to the community. Select stocks are also available from the NIG and the THFC.

**qPCR primers**

**Target** **Forward Primer** **Reverse primer**

*trk* *CCAAAATTCTGGGACAGGCAT AGATGATAGCTCTTCTCCTCGG*

*CG4953 CCACGGATTTGGTCCAGAGAT CCCAGGTAAATGCTGCCGAA*

*CG4957 AAGTACCGACTCGACATCAAGT CGCATCCATTTTAACGCCAGA*

*Mulk GGAGATCCTTCGGACGAATCA CAATGGCATCGGGTAGCGT*

*Utx TATCCTTCGACCTTTTCGGAACT GCCTTGTGCTTGTTTTGAACTTC*

*CG34043 TTTCCGTTCGTGCAAAATGAGT CCGTCGATCAAATCTCCTTGAG*

*grk GTCGCCGTCACAGATTGTTG GATTGAGCAACTCAACCGCG*

*Akap200 GGTGAGGATATAACCCCGCTG GCTTCGGAAGGACCACTTCTT*

*D12 CTTTTTGGGGACGACATAGCC CGCTCTGGCAAATGATTTTGGAT*

*Chrac-14 GCATCGAGGATCTTAACCTGC GCTGACACTAGCTGACTCCG*

*spz-6 AAGTCTGCCGTGTCCGTTC CGAGTACATTTTGTCCCAGCG*

*Usp15-31 CAGACACGAGCACGACGAAT CACTGCCGTTTTGAACTTACG*

*CG3880 TCACTCCGTCGGGTCACTC ACAGCGTTTTGCTGATTGCG*

*CG12848 CCTCAAAGCGAGAAGGACGT GAGACAGGCCACATCGGATC*

*CG3894 ATCCATACCACGGGTCCAATA CGTCGGCAAAACTAGCTCTCC*

*spz-4 CGCACCCAAATGGATAGGC GCAATCGTCATTAGGATCAGCA*

*CG6488 AGAACGAGAGGGTACTGAAGC GCGAGGTAGTTATCCAGCTCC*

*Dlg5 TACAAGTCCGCACTTTTGGAG GGTGGCTAACTCATCGCATC*

*spz GACACCTGGCAGTTAATTGTCA CGAAGTCACAGGGTTGATCCG*

*Nep5 ACGAGCAGACTGATCCCTG GAACCAATCGTTCGAGGTCAC*

*CG34292 TCTTCATGGTGGTGGCTCTAA TGCTTTTACAGTCACCTCTTGG*

*CG43935 TGGATCGCGTCGTTGGCAAT CCAACTTGCGCAGGGTCCAG*

*CG14257 AGTCCCAGGTGCAAAAGCG TCGTAGTCCACAGAGTTGCCA*

**SUPPLEMENTAL REFERENCES**

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