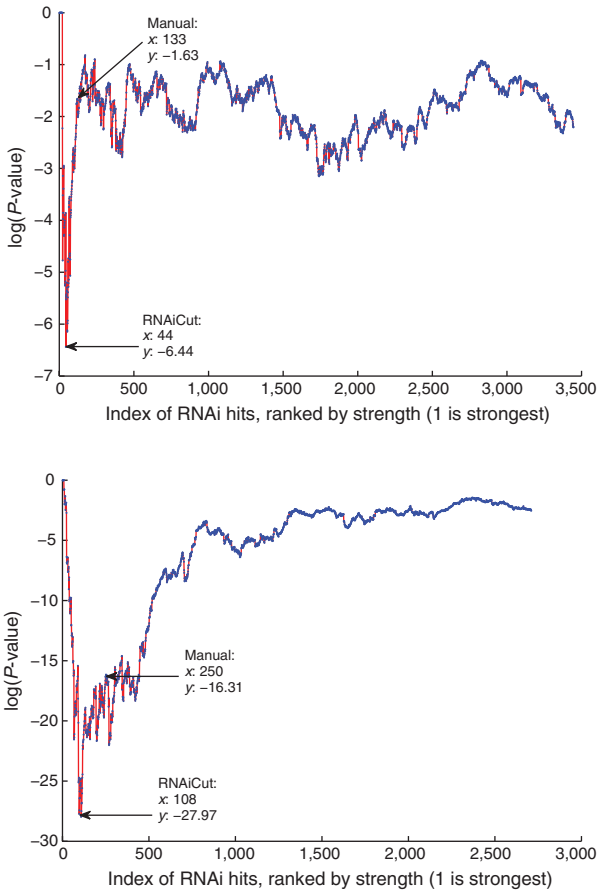

Figure 1 | RNAiCut results for insulin-triggered MAPK pathway screen in *D. melanogaster*[4]. Genes with positive (top) and negative (bottom) *Z* scores in the screen are ordered on the *x* axes from left to right based on the decreasing magnitude of *Z* scores. The *y* axis denotes the *P*-value, as a function of *k*, of finding a random PPI subnetwork as well connected as the one containing the *k* highest-scoring genes from the RNAi screen.

the core signaling pathway. RNAiCut was robust to *Z*-score noise generated by randomly scrambling close *Z* scores (**Supplementary Fig. 11** and **Supplementary Table 5**).

We offer an online server (http://rnaicut.csail.mit.edu) for interpreting functional genomic experiments. Although we developed RNAiCut using a fly PPI network, RNAiCut can also be run on non-fly and non-PPI networks (**Supplementary Fig. 12**). This tool will help functional genomics research by enabling hit-list gene selection using orthogonal datasets.

Note: Supplementary information is available on the Nature Methods website.

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Enabling IMAC purification of low abundance recombinant proteins from *E. coli* lysates

To the Editor: Currently, the most widely used method for purifying recombinant proteins for biochemical and especially structural studies is immobilized metal affinity chromatography (IMAC), in which a metal-binding polyhistidine tag (His tag) serves as a small purification handle on the target protein. IMAC is a powerful and generic purification method, with high recovery yields and low costs. Additionally, the His tag is compatible with most downstream applications because it is small and relatively inert[1,2]. *Escherichia coli* is by far the most popular expression host owing to its supremacy regarding cost, biomass production and technical simplicity[3,4]. However, a serious drawback of IMAC is the often-experienced failure to purify low-abundance His-tagged proteins from *E. coli* lysates; increasing the culture size and thereby increasing the amount of available His-tagged protein does not result in increased yield. We examined this issue and propose that it is tightly linked to metal-ion leakage from the columns induced by the *E. coli* lysate.

We used His-tagged GFP (His_6-GFP) to examine the effect of *E. coli* lysate on the protein binding capacity of IMAC columns. Application of the soluble fraction of *E. coli* lysate lacking recombinant protein expression to a 1 ml HiTrap Chelating HP column (GE Healthcare) partly loaded with His_6-GFP, caused extensive migration of His_6-GFP whereas application of wash buffer did not (**Supplementary Fig. 1a**).We confirmed this using different column materials and concluded that *E. coli* lysate severely reduces the binding capacity of the column (data not shown). By separating a lysate into high- and low-molecular-weight components we found that the reduced binding capacity was brought about by low-molecular-weight components, and not high-molecular-weight components (**Supplementary Fig. 1b**), implying that the underlying cause for the reduced target protein binding is not the result of native *E. coli* proteins competing with the His-tagged protein for the immobilized nickel-ion binding sites. We determined the amount of nickel present on the different columns before and after sample load and found that the decrease in binding capacity correlated with loss of immobilized nickel ions from the column (**Supplementary Fig. 1c**).

IMAC is very sensitive to the presence of metal chelators[1], and the *E. coli* lysate contains many unspecific weak chelators such as dicarboxylic acids from the citric acid cycle. Under stress conditions, *E. coli* can also produce highly specific metal chelators, metallophores[5]. We speculated that such metallophores, if produced, would be mainly associated with the periplasmic space of *E. coli* but not with the cytosol. We therefore hypothesized that removing the periplasmic material before cell lysis could improve His-tagged recombinant protein purification yields. We subjected *E. coli* cells to osmotic shock to remove the periplasmic material before cell lysis (**Supplementary Methods**). His_6-GFP did not migrate substantially on IMAC columns treated with lysate devoid of periplasmic

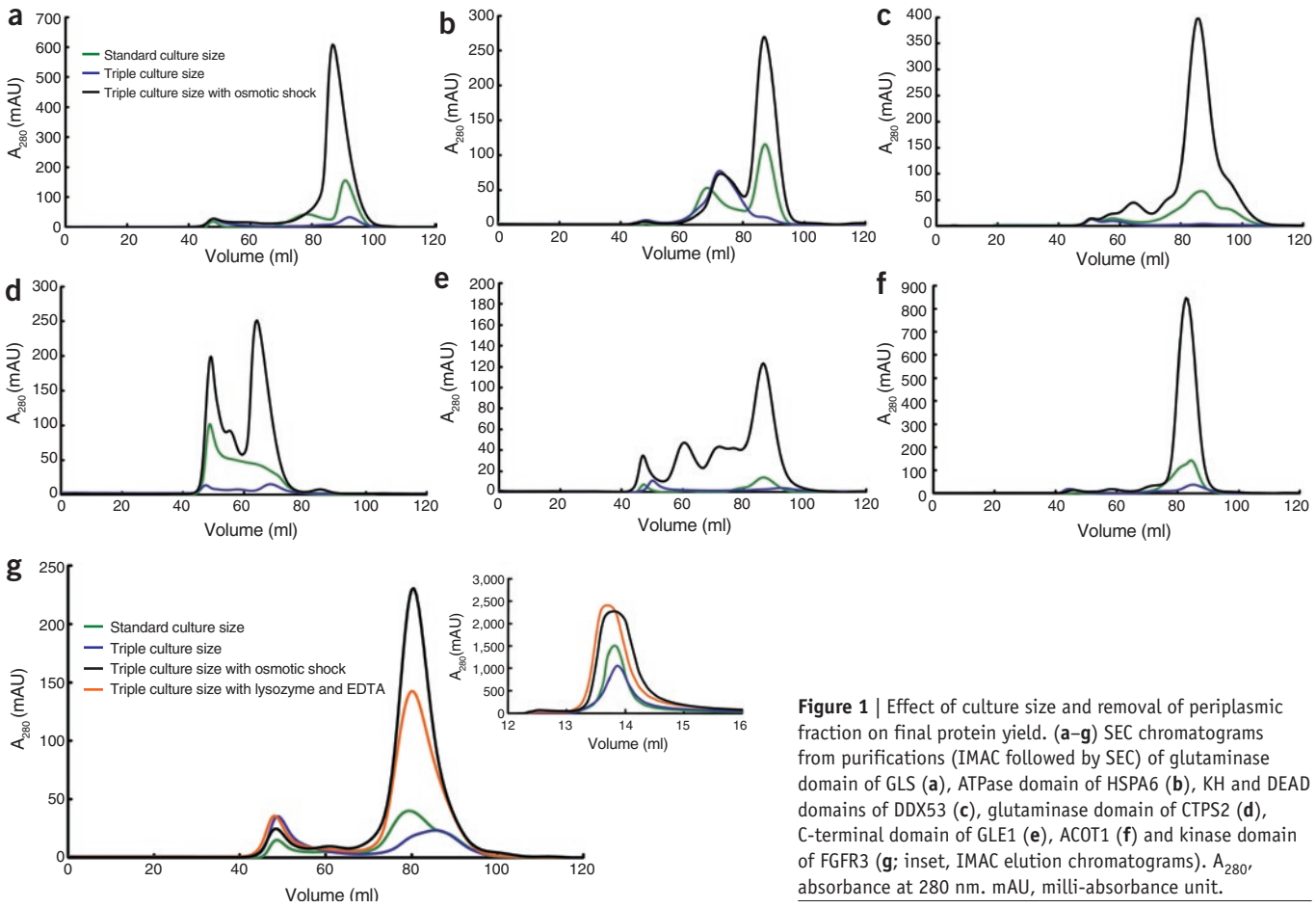

Figure 1 | Effect of culture size and removal of periplasmic fraction on final protein yield. (**a**–**g**) SEC chromatograms from purifications (IMAC followed by SEC) of glutaminase domain of GLS (**a**), ATPase domain of HSPA6 (**b**), KH and DEAD domains of DDX53 (**c**), glutaminase domain of CTPS2 (**d**), C-terminal domain of GLE1 (**e**), ACOT1 (**f**) and kinase domain of FGFR3 (**g**; inset, IMAC elution chromatograms). A_{280}, absorbance at 280 nm. mAU, milli-absorbance unit.

material (**Supplementary Fig. 1d**), suggesting that the low-molecular-weight components of *E. coli* lysate that reduce the binding capacity of IMAC columns are associated with the periplasm. In addition, spiking a lysate with 2 mM $FeSO_4$ to saturate potential metallophores also reduced the migration of prebound His_6-GFP (**Supplementary Fig. 1b**).

By removing the periplasmic material before cell lysis and loading the lysate on IMAC columns, we observed a tenfold increase in yield of His_6-GFP when it was diluted with *E. coli* lysate before purification to simulate a low-abundance protein (**Supplementary Fig. 2**). We also tested our purification protocol on seven human proteins that vary widely in abundance after heterologous overexpression (**Fig. 1** and **Supplementary Fig. 3**). We compared the resulting size exclusion chromatography (SEC) chromatograms from a standard-sized culture and two triple-sized cultures, one of which we treated with osmotic shock. Tripling the culture volume combined with osmotic shock treatment in all cases resulted in an increased yield, on average fivefold, whereas just increasing the culture size actually resulted in a decreased yield for all proteins (**Fig. 1**). We also observed the same trends when we removed the periplasmic material by lysozyme-EDTA treatment (**Fig. 1g**).

Although a considerable fraction of recalcitrant proteins will still need customized production, our findings show that it will be possible to increase the yield for many His-tagged proteins and enable IMAC purification for very low-abundance proteins. We believe that our method could greatly increase the number of recombinant proteins available for biochemical investigations. In addition, by removing the periplasmic material, a substantial scale-down of the whole purification setup, including column sizes and buffer volumes and so forth, may be possible without any loss of final yield or purity.

Note: Supplementary information is available on the Nature Methods website.

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