Since the cleavage (p.109) did not appear to go to completion an additional 20 mL of mTEVp was added to each 1 mL reaction and incubated 3.5 h. at 30°C.

The reactions were concentrated from 4 mL total to less than 1000 mL. If they TEV cleavage went to completion, this would be: (*note: Millipore 500 mL 50 MWCO filters used for conc.)

2.6 mg Cys-Pax (38-557):FLAG in < 1000 mL (2.6 mg/mL)

3 EPL reactions were run simultaneously in 0.65 mL eppendorf tubes incubated at rt overnight.

rxn a + b: (based on 54 kDa, 520 µg = 963 nmol)  
rxn C:  
520 µg Cys-Pax-FL in 200 mL (0.048 mM)  
800 µg Y31 thioester (lyophilized)  
(4 mg/mL, 0.83 mM)  
100 mM MESNA (10 µL of a 2M soln.)

100 mM MESNA

mouse anti-His 6 10 ab  
ab from Amersham  
(5 mL in 15 mL)  
for rows 1-5  
ab from Novagen  
(2.5 mL in 15 mL)  
for lane 6  
cat # 70796.4  
Kit lot # N57182

Original TEV cleavage (as on p.109)

2 TEV after additional 20 mL mTEVp
3 TEV after additional 20 mL mTEVp
3 TEV cleavages concentrated to 2.6 mg/mL
4 EPL a: For 2 gels (1 coomassie, 1 west) 20 mL protein + 20 mL 2× SDS
5 EPL b: so that’s 26 µg/gel w/ complete conversion  
(what accounting for the extra 4.8 Da)  
or 0.48 nmol/gel (from 10 µL of soln.)

(For Western blots, the nitrocellulose was blocked 1 hour w/ milk in TBST. Prior to this it was kept moist in TBS to avoid overblocking.)
Expression of GST-PTP-PEST

PTP-PEST ( ) was expressed as a GST fusion in Codon Plus RIL cells.

The protein pellet (1 g) was lysed with 40 mL of lysis buffer, as prev. described and purified with 1.5 mL of fresh GST affinity resin (Amersham).

Coomassie shows elutions 1-4 (1 mL loaded [3.5 soln, 3.5 loading buffer] per well.) 4 x 1.5 mL elutions were collected.

Dialysis into PBS (to get rid of the glutathione):
GST (only), GST-FAK, GST-GIT, and GST were dialyzed in 3 x 2L PBS in the cold room overnight.

Biorad quant.

Standards: 1 = 0.325, 2.5 = 0.472, 5 = 0.673, 7.5 = 0.808, 10 = 0.911

<table>
<thead>
<tr>
<th>GST (only) (3 mL)</th>
<th>Abs595</th>
<th>1/500 dilution</th>
<th>1/250 dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.612</td>
<td>0.777</td>
<td>1.8 mg/mL</td>
</tr>
<tr>
<td>GST-FAK (3 mL)</td>
<td>0.594</td>
<td>0.820</td>
<td>2.0 mg/mL</td>
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<tr>
<td>GST-GIT (6 mL)</td>
<td>0.623</td>
<td>0.782</td>
<td>1.8 mg/mL</td>
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<tr>
<td>GST-PEST (6 mL)</td>
<td>0.658</td>
<td>0.964</td>
<td>2.6 mg/mL</td>
</tr>
</tbody>
</table>