

**Supplementary Material for
“Src family kinase phosphorylation of the motor domain of the human kinesin-5, Eg5”**

Figure S1

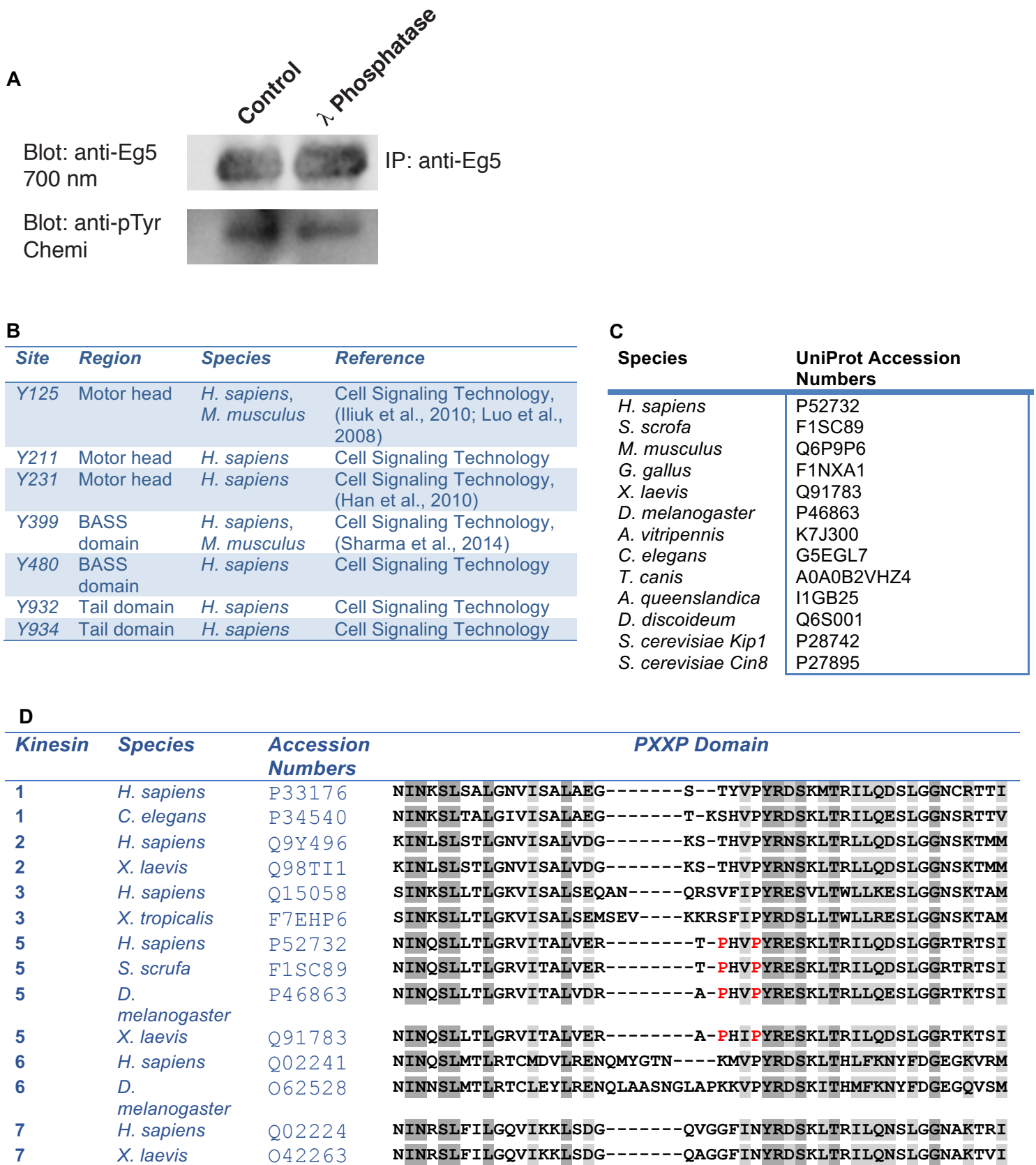


Figure S1

E

CONSTRUCT	Expression Vector	Tag	Residues	Y125	Y211	Y231	PXXP
EG5-367 E270A	pRSET	C-term 6X His	1-367	SPNEEYTWEEEDPLAG	TVHNKDEVYQIILEKGA	TAATLMNAYSSRSRSH	TALVERTPHVVPYRESKL
EG5-367 GSTY E270A	pRSET	C-term 6X His	1-367	SPNEEYTWEEEDPLAG	TVHNKDEVYQIILEKGA	TAATLMNAYSSRSRSH	TALVE GSTY VVPYRESKL
EG5-367-3Y->F E270A	pRSET	C-term 6X His	1-367	SPNEE F TWEEEDPLAG	TVHNKDEV F QIILEKGA	TAATLMNA F SSRSRSH	TALVERTPHVVPYRESKL
EG5-367-2Y->F E270A	pRSET	C-term 6X His	1-367	SPNEEYTWEEEDPLAG	TVHNKDEV F QIILEKGA	TAATLMNA F SSRSRSH	TALVERTPHVVPYRESKL
Eg5-367myc	pcDNA3	C-term Myc	1-367	SPNEEYTWEEEDPLAG	TVHNKDEVYQIILEKGA	TAATLMNAYSSRSRSH	TALVERTPHVVPYRESKL
Eg5-367myc-GSTY	pcDNA3	C-term Myc	1-367	SPNEEYTWEEEDPLAG	TVHNKDEVYQIILEKGA	TAATLMNAYSSRSRSH	TALVE GSTY VVPYRESKL
Eg5-367myc-3Y->F	pcDNA3	C-term Myc	1-367	SPNEE F TWEEEDPLAG	TVHNKDEV F QIILEKGA	TAATLMNA F SSRSRSH	TALVERTPHVVPYRESKL
Eg5-367	pRSET	C-term 6X His	1-367	SPNEEYTWEEEDPLAG	TVHNKDEVYQIILEKGA	TAATLMNAYSSRSRSH	TALVERTPHVVPYRESKL
Eg5-367 DL5	pRSET	C-term 6X His	1-367	SPNEE- - - - -LAG	TVHNKDEVYQIILEKGA	TAATLMNAYSSRSRSH	TALVERTPHVVPYRESKL
Eg5-367 GSTY	pRSET	C-term 6X His	1-367	SPNEEYTWEEEDPLAG	TVHNKDEVYQIILEKGA	TAATLMNAYSSRSRSH	TALVE GSTY VVPYRESKL
EG5-367-Y125E	pRSET	C-term 6X His	1-367	SPNEE E TWEEEDPLAG	TVHNKDEVYQIILEKGA	TAATLMNAYSSRSRSH	TALVERTPHVVPYRESKL
EG5-367-Y211E	pRSET	C-term 6X His	1-367	SPNEEYTWEEEDPLAG	TVHNKDEV E QIILEKGA	TAATLMNAYSSRSRSH	TALVERTPHVVPYRESKL
EG5-367-Y231E	pRSET	C-term 6X His	1-367	SPNEEYTWEEEDPLAG	TVHNKDEVYQIILEKGA	TAATLMNA E SSRSRSH	TALVERTPHVVPYRESKL
EG5-367-Y125F	pRSET	C-term 6X His	1-367	SPNEE F TWEEEDPLAG	TVHNKDEVYQIILEKGA	TAATLMNAYSSRSRSH	TALVERTPHVVPYRESKL
EG5-367-Y211F	pRSET	C-term 6X His	1-367	SPNEEYTWEEEDPLAG	TVHNKDEV F QIILEKGA	TAATLMNAYSSRSRSH	TALVERTPHVVPYRESKL
EG5-367-Y231F	pRSET	C-term 6X His	1-367	SPNEEYTWEEEDPLAG	TVHNKDEVYQIILEKGA	TAATLMNA F SSRSRSH	TALVERTPHVVPYRESKL
Eg5-WT-Em	Clontech	Emerald Green	1-1056	SPNEEYTWEEEDPLAG	TVHNKDEVYQIILEKGA	TAATLMNAYSSRSRSH	TALVERTPHVVPYRESKL
Eg5-GSTY-Em	Clontech	Emerald Green	1-1056	SPNEEYTWEEEDPLAG	TVHNKDEVYQIILEKGA	TAATLMNAYSSRSRSH	TALVE GSTY VVPYRESKL
Eg5-Y211E-Em	Clontech	Emerald Green	1-1056	SPNEEYTWEEEDPLAG	TVHNKDEV E QIILEKGA	TAATLMNAYSSRSRSH	TALVERTPHVVPYRESKL
Eg5-Y211F-Em	Clontech	Emerald Green	1-1056	SPNEEYTWEEEDPLAG	TVHNKDEV F QIILEKGA	TAATLMNAYSSRSRSH	TALVERTPHVVPYRESKL
Eg5-Y125E-Em	Clontech	Emerald Green	1-1056	SPNEE E TWEEEDPLAG	TVHNKDEVYQIILEKGA	TAATLMNAYSSRSRSH	TALVERTPHVVPYRESKL
Eg5-Y125F-Em	Clontech	Emerald Green	1-1056	SPNEE F TWEEEDPLAG	TVHNKDEVYQIILEKGA	TAATLMNAYSSRSRSH	TALVERTPHVVPYRESKL
Eg5-Y125E,Y211E-Em	Clontech	Emerald Green	1-1056	SPNEE E TWEEEDPLAG	TVHNKDEV E QIILEKGA	TAATLMNAYSSRSRSH	TALVERTPHVVPYRESKL

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Name	Vector	Tag	Mutations	Use
<i>Klp61F E266A</i>	pRSET	C-term 6X His tag	E266A	Bacterial expression, <i>in vitro</i> kinase assays
<i>c-Src Active</i>	pCMV-SPORT 6	(none)	Y527F	Mammalian expression, transfection experiments
<i>c-Src IR</i>	pCMV-SPORT 6	(none)	T338M, Y527F	Mammalian expression, transfection experiments

Figure S2

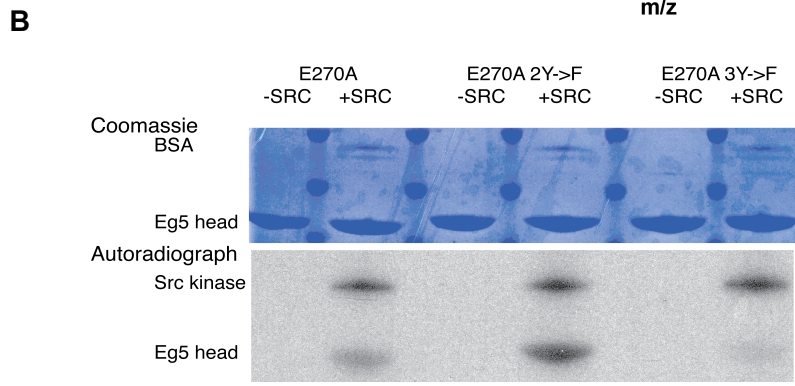
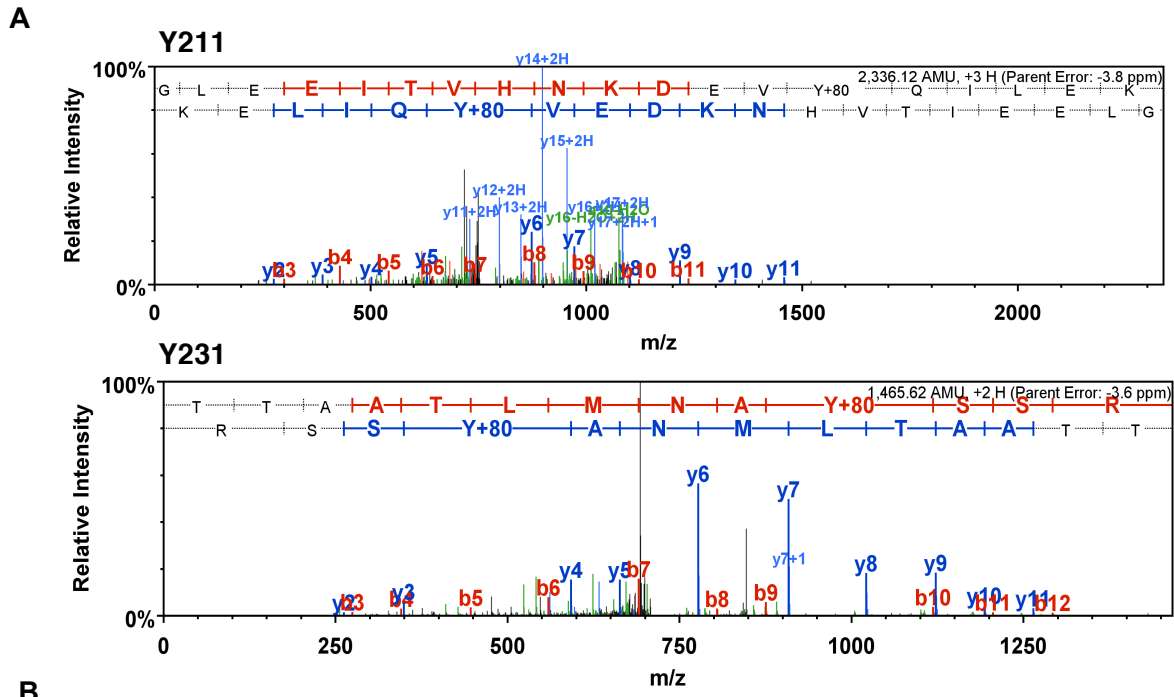
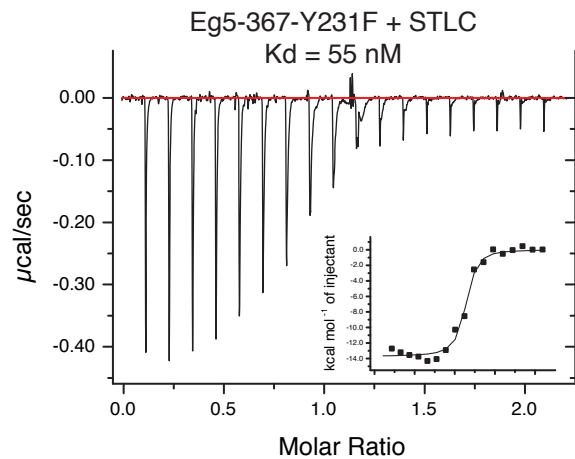
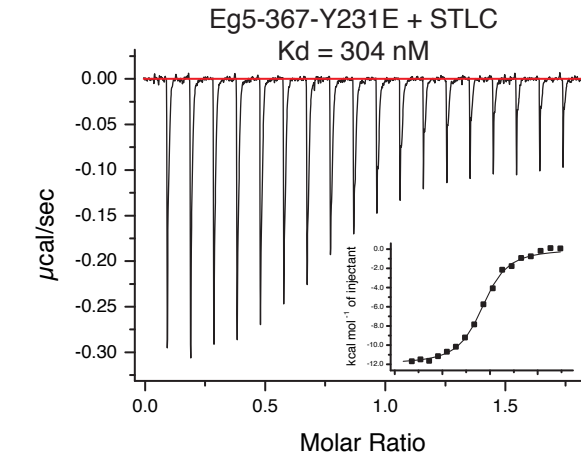
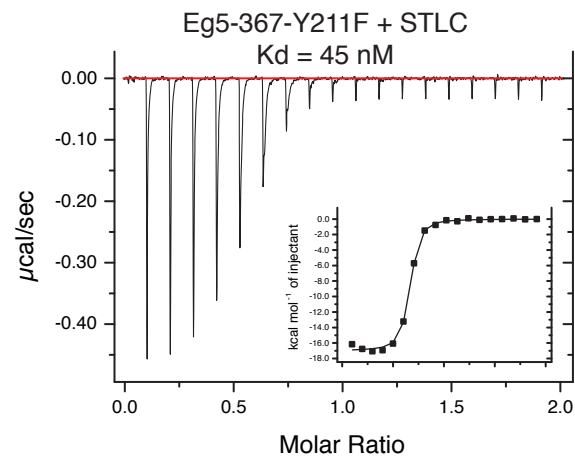
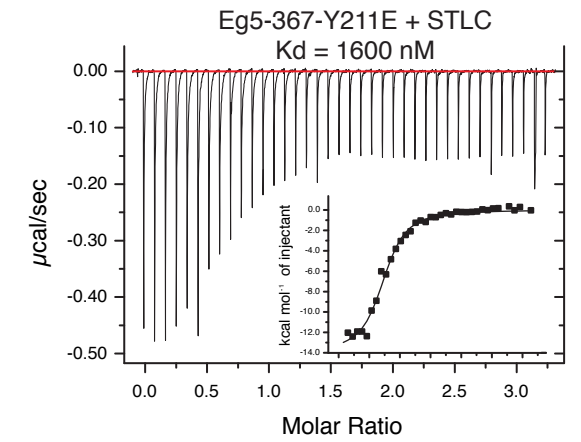
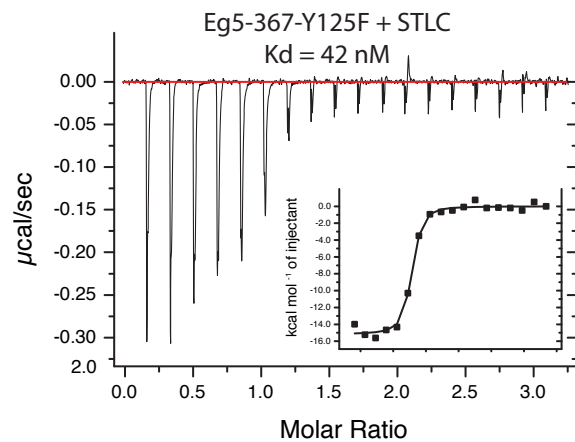
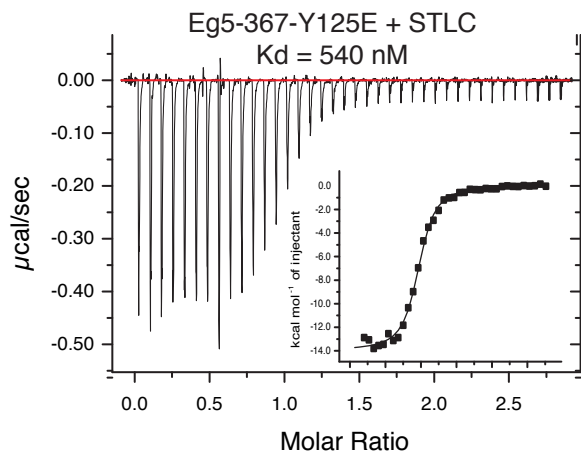
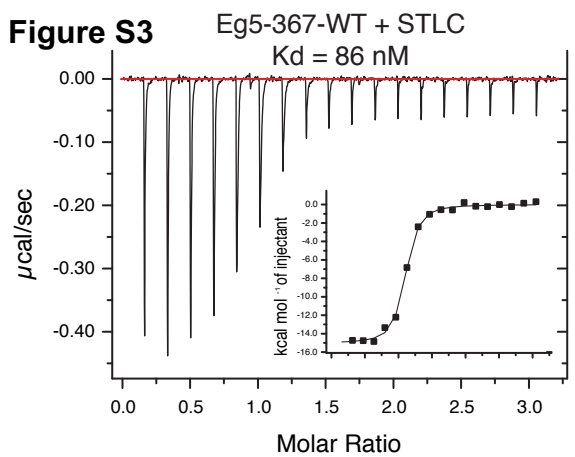


Figure S3

Protein	N	K _D (kcal mol ⁻¹)	H (kcal mol ⁻¹)	TΔS (kcal mol ⁻¹)
WT	1.07	86±21	-15.1±3.0	-5.6
Y125F	1.03	42±18	-13.4±4.3	-4.9
Y125E	0.95	540±150	-16.3±4.5	-6.4
Y211F	0.62	45±6.0	-14.0±4.2	-6.1
Y211E	0.74	1600±250	-17.0±1.3	-7.1
Y231F	1.09	55±19	-12.0±1.4	-3.2
Y231E	0.89	304±32	-13.7±2.9	-3.9

Figure S4

