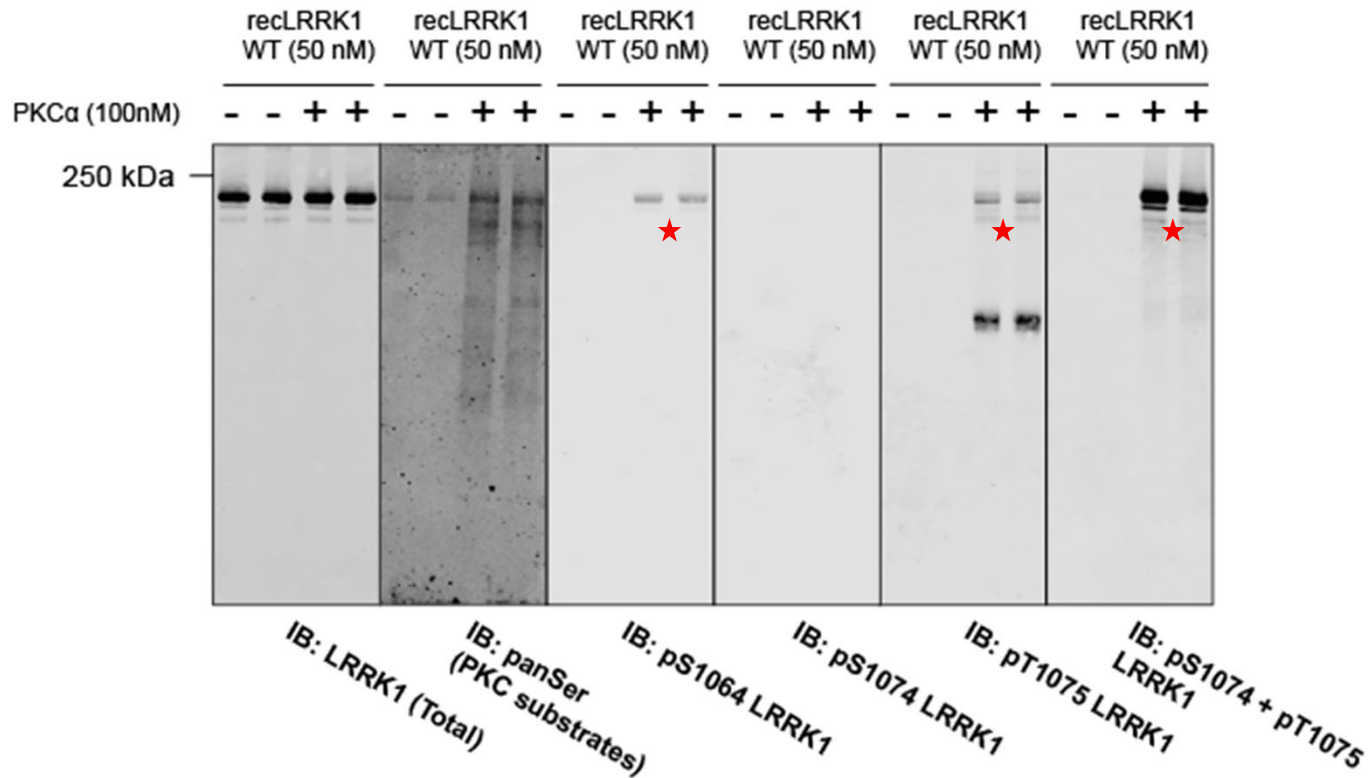


Antibody Validation:

- DA160 (α -pSer1064 LRRK1)
- DA208 (α -pSer1074 LRRK1)
- DA209 (α -pThr1075 LRRK1)
- DA210 (α -pSer1074 + pThr1075 LRRK1)

Antibody validation using recombinant LRRK1

α -panSer (PKC substrate) used as positive control for LRRK1 phosphorylation by PKC



in vitro kinase assay:

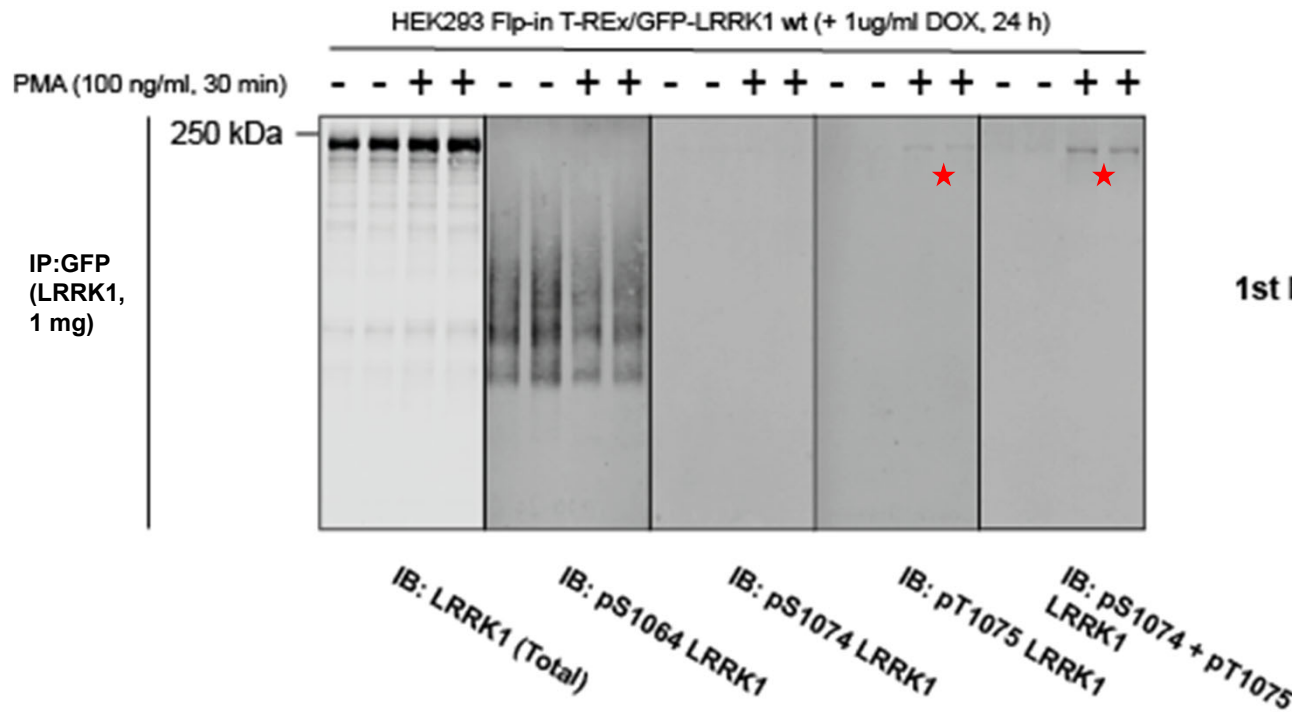
- Recombinant PKC α (100 nM)
- Recombinant LRRK1 wt (50 nM)
- 1 mM ATP
- 25% of reaction loaded onto gel (**100ng** of recombinant LRRK1)

Only α -pSer1074 LRRK1 antibody is unable to detect signal

All antibodies tested at 1 μ g/ml

Antibody validation using immunoprecipitated LRRK1

Phorbol ester (PMA) treatment used to induce LRRK1 phosphorylation in cells



GFP-LRRK1 immunoprecipitated from 1 mg of stable overexpressing cell line extracts:

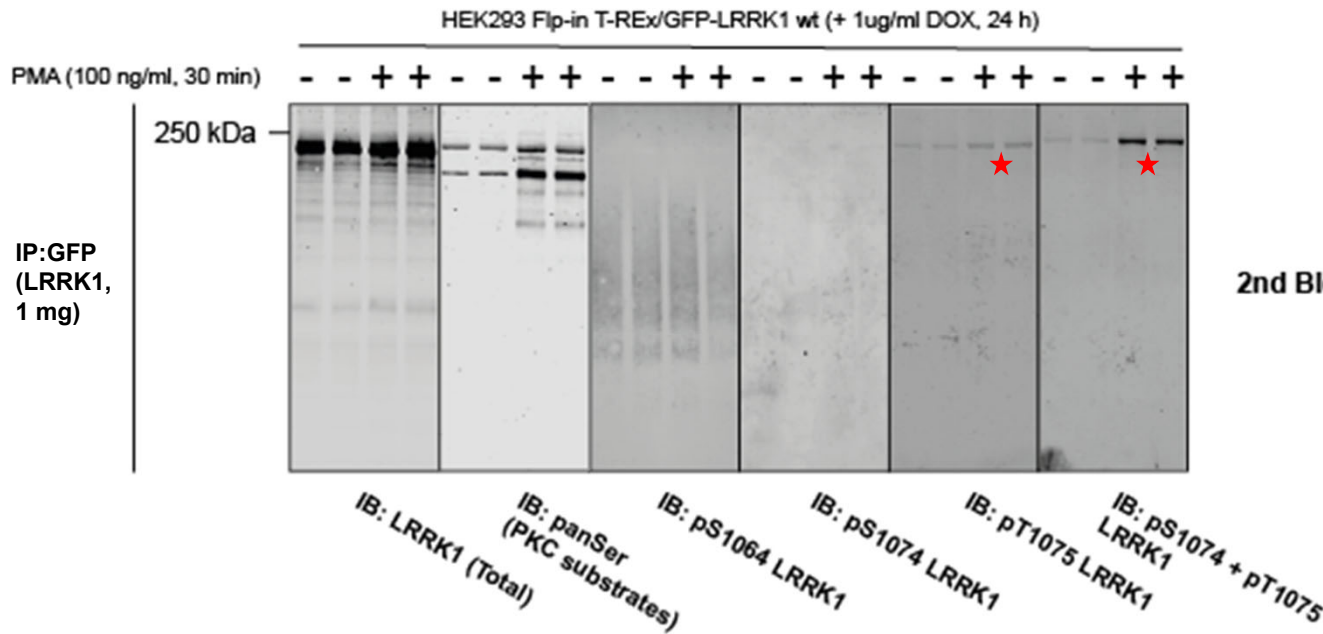
- Complete IP (**1 mg**) loaded onto gel

Only α -pThr1075 LRRK1 (weak) and α -pSer1074 + pThr1075 LRRK1 antibodies are able to detect signal in IPs

All antibodies tested at 1 μ g/ml

Antibody validation using immunoprecipitated LRRK1

Phorbol ester (PMA) treatment used to induce LRRK1 phosphorylation in cells



GFP-LRRK1 immunoprecipitated from 1 mg of stable overexpressing cell line extracts:

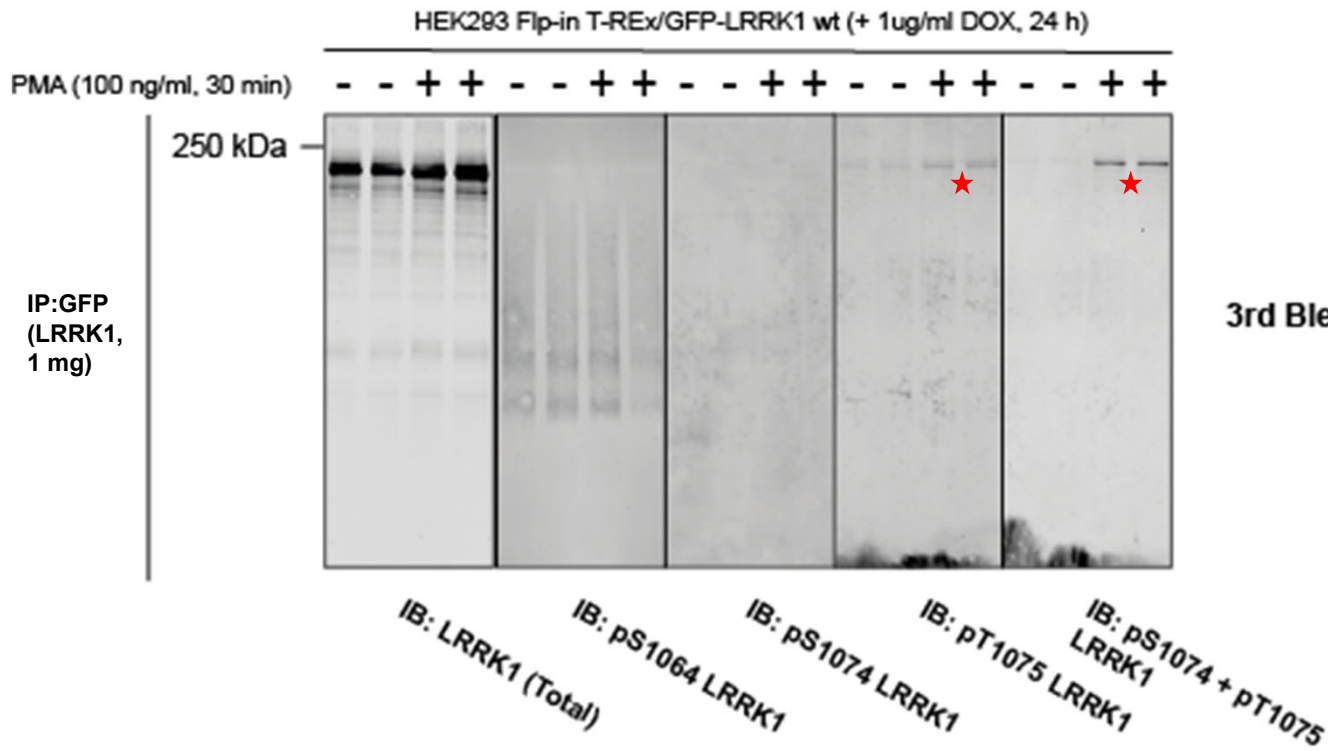
- Complete IP (1 mg) loaded onto gel

Only α -pThr1075 LRRK1 (weak) and α -pSer1074 + pThr1075 LRRK1 antibodies are able to detect signal in IPs

All antibodies tested at 1 μ g/ml

Antibody validation using immunoprecipitated LRRK1

Phorbol ester (PMA) treatment used to induce LRRK1 phosphorylation in cells



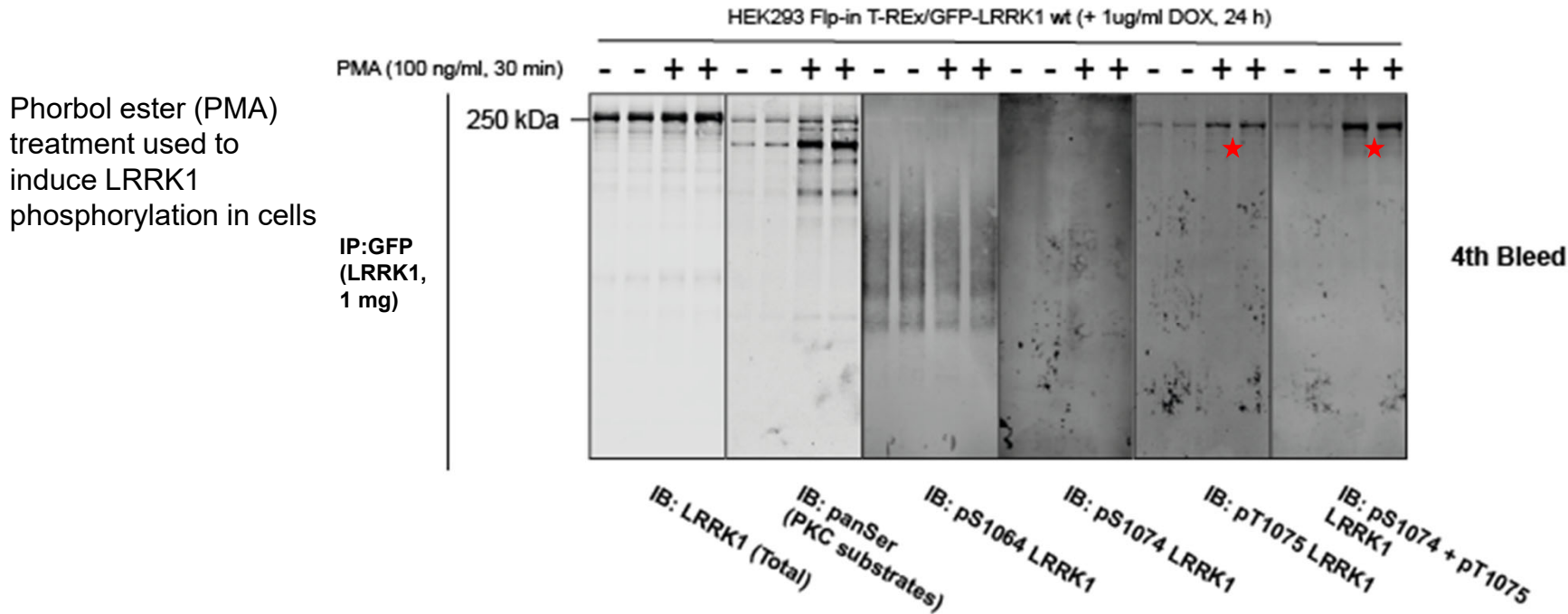
GFP-LRRK1 immunoprecipitated from 1 mg of stable overexpressing cell line extracts:

- Complete IP (1 mg) loaded onto gel

Only α -pThr1075 LRRK1 (weak) and α -pSer1074 + pThr1075 LRRK1 antibodies are able to detect signal in IPs

All antibodies tested at 1 μ g/ml

Antibody validation using immunoprecipitated LRRK1



GFP-LRRK1 immunoprecipitated from 1 mg of stable overexpressing cell line extracts:

- Complete IP (1 mg) loaded onto gel

Only α -pThr1075 LRRK1 (weak) and α -pSer1074 + pThr1075 LRRK1 antibodies are able to detect signal in IPs

α -pSer1074 + pThr1075 LRRK1 taken forward for testing in cell lysates

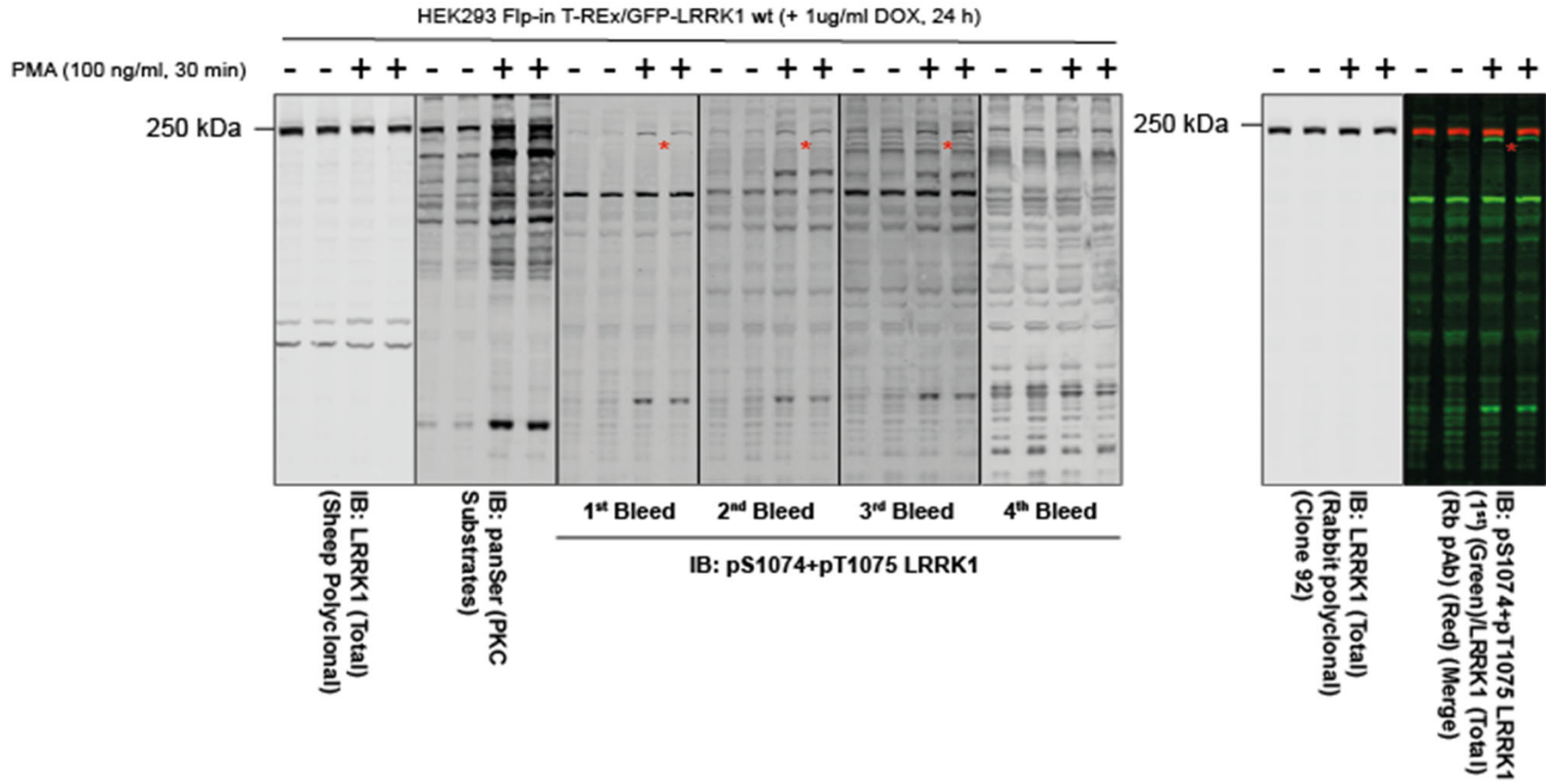
All antibodies tested at 1 μ g/ml

Antibody validation using cell lysates

Phorbol ester (PMA) treatment used to induce LRRK1 phosphorylation in cells

GFP-LRRK1 stably overexpressing cell line:
 • **20 µg** of cell lysate loaded onto gel

*** = Unclear if this corresponds to LRRK1 species as it migrates slightly lower than total protein**



All antibodies tested at 1 µg/ml

Summary

- α -pSer1064, α -pThr1075 and α -pSer1074 + pThr1075 able to detect signal *in vitro*
- α -pSer1074 + pThr1075 able to detect in IPs
 - α -pThr1075 detects weakly in IPs
- Unclear if α -pSer1074 + pThr1075 is viable for use against cell lysates