## Expression of Syk and MAP4 proteins in ovarian cancer

Siwei Zhang<sup>1</sup>, Suha Deen<sup>2</sup>, Sarah J Storr<sup>1</sup>, Anqi Yao<sup>1</sup>, Stewart G Martin<sup>1,3</sup>

 Academic Clinical Oncology, Nottingham Breast Cancer Research Centre, School of Medicine, University of Nottingham, Nottingham University Hospitals NHS Trust, City Hospital Campus, Nottingham, NG5 1PB, UK.
Department of Pathology, Queen's Medical Centre, Nottingham University Hospital, Nottingham, NG7 2UH, UK
Corresponding author: Academic Clinical Oncology, Nottingham Breast Cancer Research Centre, School of Medicine, University of Nottingham, Nottingham University Hospitals NHS Trust, City Hospital Campus, Nottingham, NG5 1PB, UK

Telephone: +44(0)115 823 1846

Email: stewart.martin@nottingham.ac.uk.

## **Supplements**

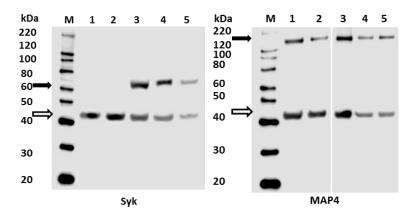


Fig. S1 The specificity of anti-Syk and anti-MAP4 antibody. Syk and MAP4 expression across the panel of ovarian cancer cell lines were detected by Western blotting analysis. Primary antibodies were diluted in 1:1000 dilution. A single band corresponding to the molecular mass of the intended target was observed in the Western blotting studies which indicated the specificity of both anti-MAP4 (BETHYL LABORATORIES, UK) and anti-Syk (Thermo Fisher SCIENTIFIC, UK) antibody. Figures present the representative blots of 2 independent experiments with lysates from cells with different passage numbers. Open arrows indicate  $\beta$ -actin (42 kDa) which was used as the loading control. Black arrows indicate Syk (72 kDa) and MAP4 (210 kDa), respectively. Lane M: Protein marker, Lane 1: A2780, Lane 2: A2780-cis, Lane 3: SKOV3, Lane 4: PEO1, and Lane 5: PEO4.

| Variable                     | Syk-c      |             |         | Syk-n       |             |         | MAP4        |             |          |
|------------------------------|------------|-------------|---------|-------------|-------------|---------|-------------|-------------|----------|
|                              | Low        | High        | P-value | Low         | High        | P-value | Low         | High        | P-value  |
| Stage                        |            |             |         |             |             |         |             |             |          |
| Tumour confined to ovaries   | 36(8.3%)   | 123 (28.3%) | 0.002   | 50 (11.5%)  | 109 (25.1%) | 0.041   | 75 (17.2%)  | 89 (20.5%)  | 0.000087 |
| (stage 1)                    |            |             |         |             |             |         |             |             |          |
| Tumour spread out of ovaries | 31 (7.1%)  | 244 (56.2%) |         | 62 (14.3%)  | 213 (49.1%) |         | 74 (17.0%)  | 197 (45.3%) |          |
| (stage 2, 3 and 4)           |            |             |         |             |             |         |             |             |          |
| Stage                        |            |             |         |             |             |         |             |             |          |
| Without distant metastasis   | 62 (14.3%) | 341 (78.6%) | 0.912   | 104 (24.0%) | 299 (68.9%) | 1.000   | 139 (32.0%) | 265 (60.9%) | 0.808    |
| (stage 1,2 and 3)            |            |             |         |             |             |         |             |             |          |
| Distant metastasis occurred  | 5 (1.2%)   | 26 (6.0%)   |         | 8 (1.8%)    | 23 (5.3%)   |         | 10 (2.3%)   | 21 (4.8%)   |          |
| (stage 4)                    |            |             |         |             |             |         |             |             |          |

## Table S1 The association between protein expression and tumour stage

Significant *P* values are indicated by bold font.

| Variables   | P value |                 |             |  |  |  |
|---|---------|-----------------|-------------|--|--|--|
|   | MAP4    | Cytoplasmic Syk | Nuclear Syk |  |  |  |
| Histological subtypes                               |         |                 |             |  |  |  |
| High grade serous carcinoma                         | 0.699.  | 0.581           | 0.506       |  |  |  |
| Mucinous carcinoma                                  | 0.791   | 0.026           | 0.115       |  |  |  |
| Endometrioid carcinoma                              | 0.110   | 0.188           | 0.006       |  |  |  |
| Clear-cell carcinoma                                | 0.988   | 0.822           | 0.453       |  |  |  |
| Low grade serous carcinoma                          | 0.212   | 0.549           | 0.196       |  |  |  |
| Stage   |         |                 |             |  |  |  |
| Stage 1 (confined tumour) (n=152)                   | 0.679   | 0.002           | 0.001       |  |  |  |
| Stage 2, 3 & 4 (tumour spread) (n=269)              | 0.404   | 0.688           | 0.224       |  |  |  |
| Stage   |         |                 |             |  |  |  |
| Stage 1, 2 & 3 (without distant metastasis) (n=390) | 0.104   | 0.568           | 0.107       |  |  |  |
| Stage 4 (with distant metastasis) (n=31)            | 0.061   | 0.402           | 0.509       |  |  |  |
| Sensitivity to platinum-based chemotherapy          |         |                 |             |  |  |  |
| Sensitive (n=195)                                   | 0.885   | 0.486           | 0.464       |  |  |  |
| Resistant (n=40)                                    | 0.996   | 0.016           | 0.001       |  |  |  |
| Residual disease                                    |         |                 |             |  |  |  |
| No residual tumour (n=232)                          | 0.867   | 0.054           | 0.001       |  |  |  |
| residual tumour (n=147)                             | 0.540   | 0.349           | 0.571       |  |  |  |

Table S2 P values from the log-rank test of overall survival in different subgroups

Significant *P* values are indicated by bold font.

Table S3 P values from the log-rank test of overall survival related to combinational protein expression.

| •                        |                    |                    |                    |                    |                    |
|--------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|                          | Syk-c              | Syk-n              | MAP4               | CXCL4              | CXCR3              |
|                          | ( <i>P=</i> 0.494) | ( <i>P</i> =0.113) | ( <i>P=</i> 0.064) | ( <i>P=</i> 0.031) | ( <i>P=</i> 0.182) |
| Calpastatin              | 0.174              | 0.178              | 0.024              | 0.225              | 0.188              |
| ( <i>P=</i> 0.010)       |                    |                    |                    |                    |                    |
| Calpain-1                | 0.273              | 0.292              | 0.283              | 0.309              | 0.445              |
| ( <i>P=</i> 0.153)       |                    |                    |                    |                    |                    |
| Calpain-2                | 0.087              | 0.056              | 0.037              | 0.285              | 0.152              |
| ( <i>P=</i> 0.026)       |                    |                    |                    |                    |                    |
| Calpain-4                | 0.139              | 0.061              | 0.034              | 0.074              | 0.068              |
| ( <i>P=</i> 0.003)       |                    |                    |                    |                    |                    |
| Syk-c ( <i>P</i> =0.494) | ١                  | 0.424              | 0.220              | 0.358              | 0.468              |
| Syk-n ( <i>P=</i> 0.113) | \                  | \                  | 0.112              | 0.166              | 0.208              |
| MAP4 ( <i>P</i> =0.064)  | 1                  | \                  | ١                  | 0.084              | 0.097              |