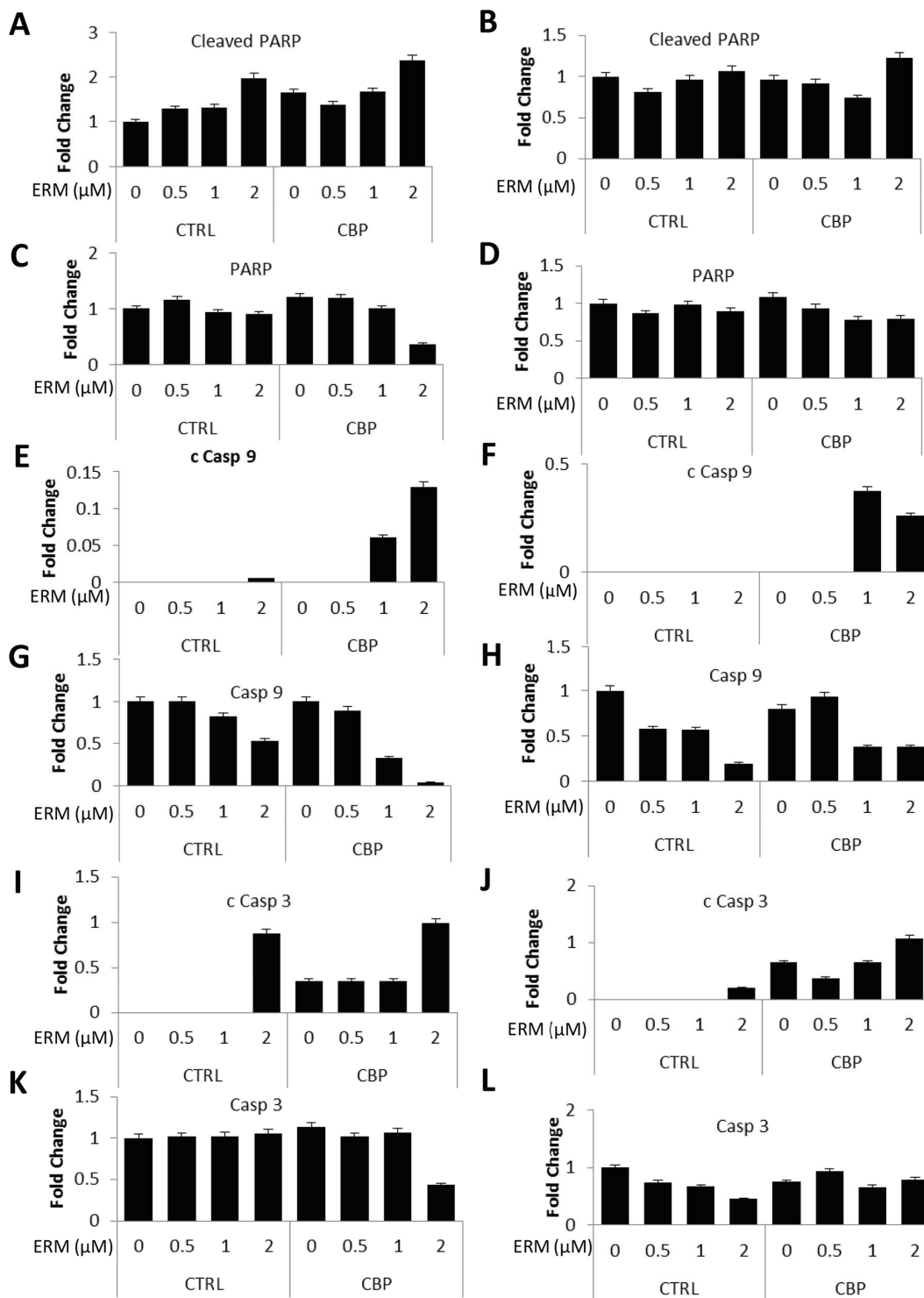
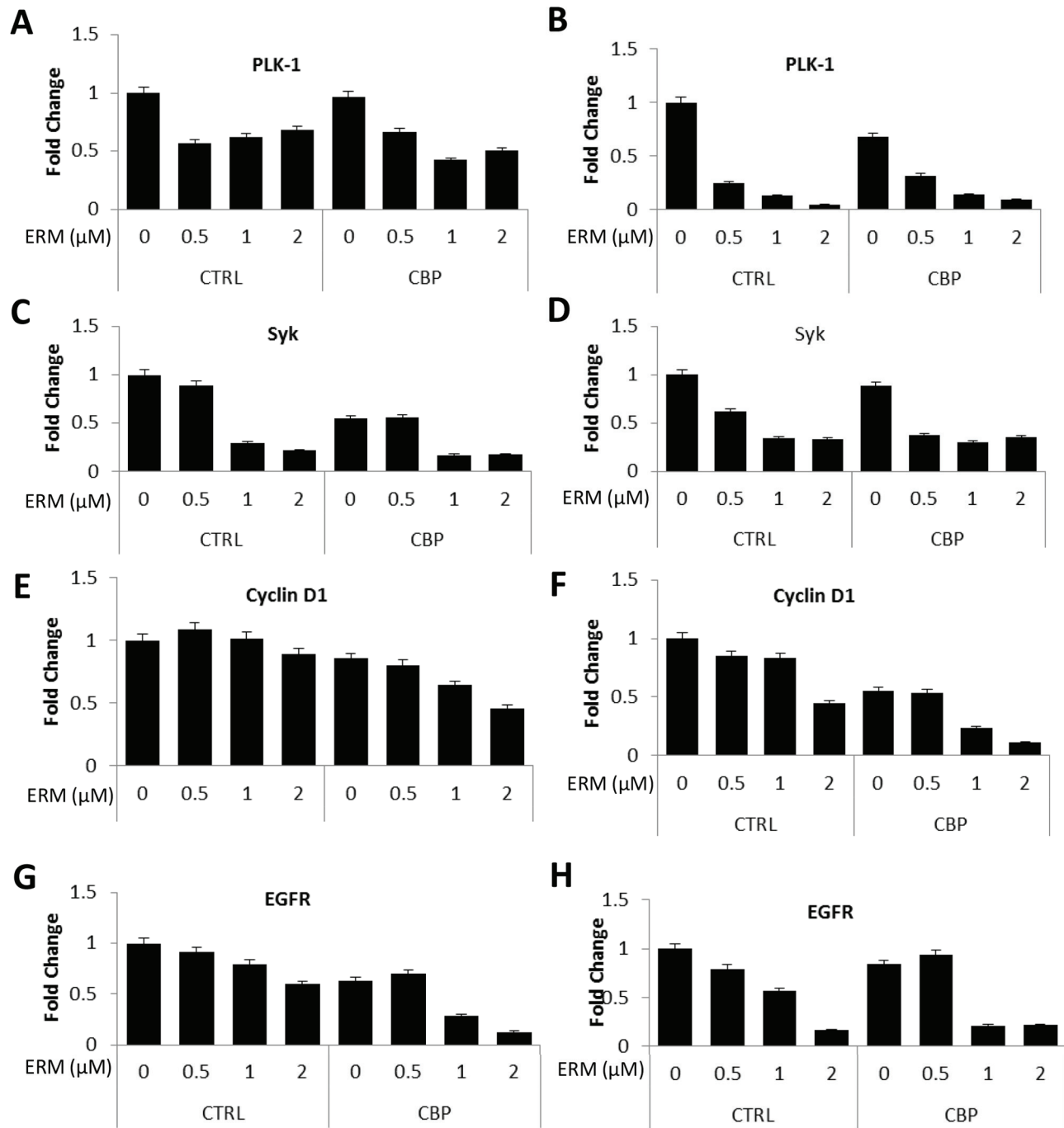


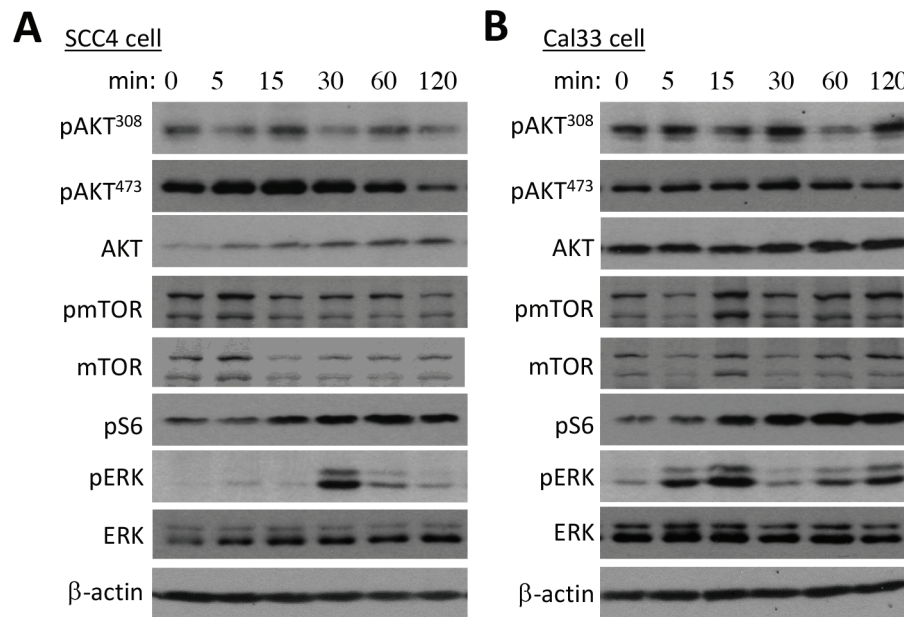
SUPPLEMENTARY FIGURES AND TABLES



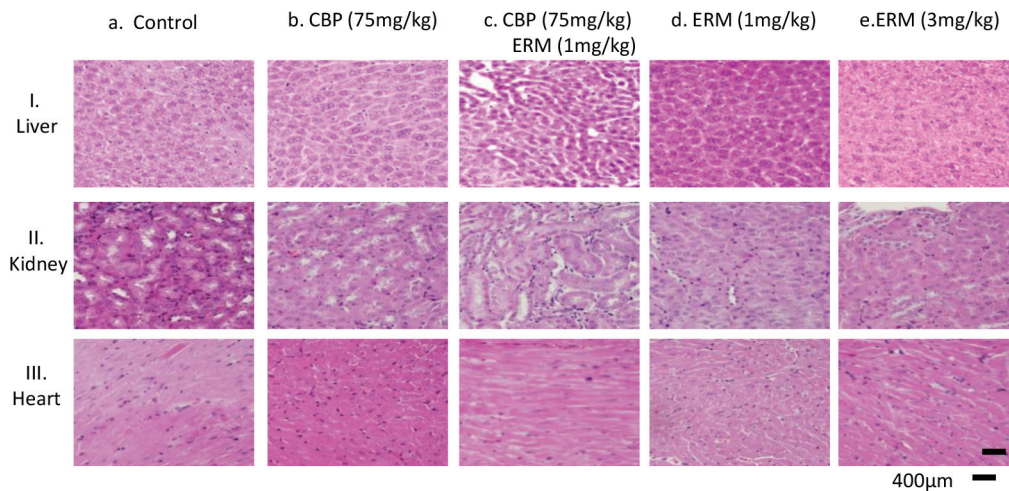
Supplementary Figure S1: Western blot densitometry analysis. Histograms of the western blot densitometry analysis of protein expression in SCC4 or Cal33 cells on treatment of ER maleate with or without presence of carboplatin (CBP 25μM). All the protein expression levels were normalised to GAPDH in comparison to untreated controls (NTC). **A.** Cleaved PARP in SCC4 cell; **B.** Cleaved Caspase 9 in Cal33 cell; **C.** PARP in SCC4 cells; **D.** PARP in Cal33 cell; **E.** Cleaved Caspase 9 in SCC4 cell; **F.** Caspase 9 in Cal33 cell; **G.** Caspase 9 in SCC4 cells; **H.** Caspase 9 in Cal33 cell; **I.** Cleaved Caspase 3 in SCC4 cell; **J.** Caspase 3 in Cal33 cell; **K.** Caspase 3 in SCC4 cells; **L.** Caspase 3 in Cal33 cell.



Supplementary Figure S2: Western blot densitometry analysis. Histograms of the western blot densitometry analysis of protein expression in SCC4 or Cal33 cells on treatment of ER maleate with or without presence of carboplatin (CBP 25 μ M). All the protein expression levels were normalised to GAPDH in comparison to untreated controls (NTC). **A.** PLK1 in SCC4 cell; **B.** PLK1 in Cal33 cell; **C.** Syk in SCC4 cells; **D.** Syk in Cal33 cell; **E.** Cyclin D1 in SCC4 cell; **F.** Cyclin D1 in Cal33 cell; **G.** EGFR in SCC4 cells; **H.** EGFR in Cal33 cell.



Supplementary Figure S3: ER maleate inhibits PI3K/Akt/mTOR and pERK signaling in OSCC cells. SCC4 and Cal33 cells were treated with ER maleate (2 μM) for a short period (5-120 min). ER maleate treatment reduced the levels of pAkt⁴⁷³ and pAkt³⁰⁸ in SCC4 **A.** and Cal33 cells **B.** Similarly, phosphorylated mTOR (pmTOR) was also decreased whereas pS6 increased in both cells (A, B). On contrary, the activity of phosphorylated ERK (pERK) was induced by ER maleate in both cells (A, B). β-actin served as a loading control.



Supplementary Figure S4: ER maleate anticancer potential in tumor xenograft mice model. Hematoxylin and eosin stained liver, kidney and heart tissue sections. Histology of liver (I), kidney (II) and heart (III) tissues obtained at conclusion of the *in vivo* study. Hematoxylin-eosin (H&E) staining showed normal histology with no obvious signs of oncoytic necrosis or fibrosis observed in tissue sections (a-e) from different treatment groups. Original magnification is 400x.

Supplementary Table S1: ER maleate (ERM) effect on cell cycle in SCC4 cells using FACS and modfit analysis

| SCC4\ERM | 0 μ M | | 0.5 μ M | | 1 μ M | | 2 μ M | |
|--------------------|-----------|--------|-------------|--------|-----------|--------|-----------|--------|
| Dip G ₁ | | 46.11% | | 43.25% | | 25.18% | | 16.56% |
| Dip G ₂ | 95.42% | 15.37% | 93.88% | 13.56% | 82.84% | 21.63% | 70.82% | 43.44% |
| Dip S | | 38.52% | | 43.19% | | 53.18% | | 40.00% |
| Anu G ₁ | | 37.98% | | 0 | | 0 | | 0 |
| Anu G ₂ | 4.58% | 0 | 6.12% | 0.54% | 17.16% | 0.05% | 29.18% | 0.32% |
| Anu S | | 62.05% | | 99.46% | | 99.95% | | 99.68% |

Dip G₁: Diploid G₁ phase; Dip G₂: Diploid G₂ phase; Dip S: Diploid S phase; Anu G₁: Polyploid G₁ phase; Anu G₂: Polyploid G₂ phase; Anu S: Polyploid S phase

Supplementary Table S2: ER maleate (ERM) effect on cell cycle in Cal33 cells using FACS and modfit analysis

| Cal 33\ERM | 0 μ M | | 0.5 μ M | | 1 μ M | | 2 μ M | |
|--------------------|-----------|--------|-------------|--------|-----------|--------|-----------|--------|
| Dip G ₁ | | 62.09% | | 56.17% | | 62.42% | | 59.23% |
| Dip G ₂ | 100% | 10.26% | 100% | 10.94% | 94.43% | 8.0% | 43.86% | 8.0% |
| Dip S | | 27.85% | | 32.89% | | 29.58% | | 32.77% |
| Anu G ₁ | | n/a | | n/a | | 43.55% | | 60.65% |
| Anu G ₂ | 0 | n/a | 0 | n/a | 5.57% | 5.15% | 56.14% | 6.08% |
| Anu S | | n/a | | n/a | | 51.3% | | 33.27% |

Dip G₁: Diploid G₁ phase; Dip G₂: Diploid G₂ phase; Dip S: Diploid S phase; Anu G₁: Polyploid G₁ phase; Anu G₂: Polyploid G₂ phase; Anu S: Polyploid S phase

Supplementary Table S3: Clinical chemistry profiles among different mice groups with ER maleate or carboplatin treatment

| | TEST | UNIT | DYNAMIC RANGE | | NTC | | ER 0.1mg/kg then CBP 75mg/kg | | ER 0.3mg/kg then ER 1mg/kg and CBP 75mg/kg | | ER 1mg/kg | | ER 3mg/kg | |
|-------|----------------------------|-------|---------------|------|--------|--------|------------------------------|-------|--|-------|-----------|--------|-----------|-------|
| | | | Low | High | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| ALB | Albumin | mg/dL | 15 | 60 | 25.10 | 0.94 | 22.50 | 2.33 | 21.17 | 1.05 | 24.60 | 0.57 | 25.57 | 0.91 |
| ALP | Alkaline Phosphatase | U/L | 5 | 1500 | 26.95 | 4.62 | 24.13 | 2.90 | 15.57 | 2.42 | 24.15 | 2.05 | 30.43 | 1.81 |
| ALT | Alanine Aminotransferase | U/L | 3 | 500 | 23.70 | 4.30 | 24.13 | 6.45 | 31.37 | 24.04 | 127.75 | 96.94 | 21.73 | 2.15 |
| AST | Aspartate Aminotransferase | U/L | 3 | 1000 | 136.55 | 126.88 | 153.70 | 40.41 | 147.43 | 83.11 | 286.50 | 157.26 | 97.67 | 21.91 |
| BUN | Urea Nitrogen | mg/dL | 2 | 130 | 25.08 | 5.17 | 24.77 | 2.06 | 20.40 | 2.45 | 24.45 | 1.91 | 31.10 | 0.53 |
| CREAT | Creatinine | mg/dL | 0.2 | 25 | 0.18 | 0.03 | 0.16 | 0.04 | 0.16 | 0.03 | 0.20 | 0.04 | 0.15 | 0.03 |
| B/C | BUN/Creatinine ratio** | - | 0.08 | 650 | 135.70 | 12.68 | 158.98 | 26.16 | 127.96 | 30.38 | 126.04 | 18.08 | 205.68 | 37.87 |
| TP | Protein, Total | mg/dL | 30 | 120 | 44.50 | 2.29 | 43.23 | 2.29 | 44.10 | 3.22 | 43.85 | 1.91 | 44.53 | 2.06 |
| CHOL | Cholesterol | mg/dL | NA | NA | 118.05 | 13.03 | 119.50 | 8.87 | 128.57 | 19.38 | 122.70 | 8.63 | 117.40 | 7.99 |
| HDL | High Density Lipoprotein | mg/dL | NA | NA | 68.65 | 12.26 | 66.43 | 3.80 | 59.87 | 4.01 | 67.75 | 1.91 | 74.57 | 7.22 |
| TRIG | Triglyceride | mg/dL | NA | NA | 66.45 | 6.05 | 93.17 | 22.38 | 52.33 | 12.06 | 88.45 | 25.81 | 124.80 | 8.02 |
| TBIL | Bilirubin, Total | mg/dL | 0.1 | 30 | 0.31 | 0.14 | 0.21 | 0.04 | 0.16 | 0.03 | 0.23 | 0.01 | 0.22 | 0.02 |
| GLU | Glucose | mg/dL | NA | NA | 205.35 | 72.81 | 205.97 | 40.93 | 253.33 | 41.19 | 275.65 | 49.29 | 160.73 | 6.32 |
| CA | Calcium, Total | mg/dL | 4 | 18 | 9.81 | 0.61 | 9.93 | 0.40 | 10.88 | 1.18 | 9.34 | 0.75 | 11.63 | 1.24 |
| PHOS | Phosphorus | mg/dL | 1 | 20 | 8.41 | 2.14 | 6.56 | 2.73 | 6.35 | 1.42 | 7.27 | 2.31 | 6.02 | 1.22 |
| NA | Sodium | mEq/L | 50 | 200 | 150.13 | 5.86 | 148.70 | 4.42 | 146.80 | 10.78 | 130.30 | 3.68 | 144.83 | 10.83 |
| K | Potassium | mEq/L | 1 | 10 | 5.13 | 0.67 | 5.18 | 0.73 | 4.37 | 0.55 | 4.92 | 0.05 | 4.41 | 0.72 |
| CL | Chloride | mEq/L | 50 | 200 | 109.45 | 3.93 | 112.83 | 3.36 | 109.60 | 5.60 | 98.50 | 4.53 | 105.30 | 8.93 |
| NA/K | Sodium/Potassium ratio** | - | 5 | 200 | 29.57 | 3.47 | 28.99 | 3.05 | 33.80 | 3.04 | 26.52 | 1.02 | 33.29 | 4.82 |

Supplementary Table S4: Hematology test among different mice groups with ER maleate or carboplatin treatment

| Test | Reference | | NTC | | ER 0.1mg/ kg then CBP 75mg/kg | | ER 0.3mg/kg then ER 1mg/ kg and CBP 75mg/kg | | ER 1mg/kg | | ER 3mg/kg | |
|------------------------------|--|-------------|--------|--------|-------------------------------------|-------|--|-------|-----------|-------|-----------|--------|
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| RBC (10 ¹² /L) | Red blood cells | 7-13 | 7.16 | 0.54 | 4.91 | 1.13 | 6.47 | 1.00 | 6.12 | 1.09 | 7.25 | 0.21 |
| Hgb (g/L) | Hemoglobin Concentration | 13- 16.9 | 103.75 | 7.63 | 77.33 | 17.95 | 103.00 | 8.72 | 89.00 | 12.73 | 108.00 | 4.76 |
| HCT (L/L) | Hematocrit | 0.474 | 0.37 | 0.02 | 0.27 | 0.06 | 0.38 | 0.03 | 0.32 | 0.08 | 0.38 | 0.01 |
| MCV (fL) | Mean Corpuscular Volume | 56 | 51.60 | 0.87 | 55.50 | 1.77 | 59.10 | 5.57 | 51.75 | 3.75 | 52.58 | 0.32 |
| MCH (pg/ cell) | Mean Corpuscular Hemoglobin | 15.4 | 14.48 | 0.05 | 15.73 | 0.23 | 16.03 | 1.33 | 14.60 | 0.57 | 14.90 | 0.22 |
| MCHC (g/L) | Mean Corpuscular Hemoglobin Concentration | 275 | 280.75 | 3.59 | 283.67 | 13.61 | 272.00 | 4.36 | 282.50 | 30.41 | 283.50 | 3.70 |
| PLT (10 ⁹ /L) | Platelet Count | 350 | 478.50 | 440.99 | 185.67 | 60.17 | 404.67 | 59.37 | 190.50 | 26.16 | 603.25 | 166.48 |
| WBC (10 ⁹ /L) | White Blood Cell | 5-12 | 1.16 | 0.80 | 0.97 | 0.46 | 4.38 | 3.20 | 0.45 | 0.01 | 1.80 | 1.05 |
| MPV (fL) | Mean Platelet Volume | 351 | 5.70 | 1.00 | 6.40 | 0.46 | 5.67 | 0.46 | 6.95 | 0.35 | 4.93 | 0.28 |
| RDW (%) | RBC Distribution Width | 22 | 19.05 | 0.64 | 23.80 | 1.55 | 24.30 | 3.42 | 19.75 | 1.34 | 20.03 | 0.15 |
| NE (10 ⁹ /L) | Neutrophil Count | NA | 0.47 | 0.43 | 0.28 | 0.31 | 2.97 | 2.16 | 0.12 | 0.06 | 1.21 | 0.90 |
| LY (10 ⁹ /L) | Lymphocyte Count | 10.5 | 0.44 | 0.17 | 0.48 | 0.31 | 0.72 | 0.46 | 0.25 | 0.01 | 0.39 | 0.10 |
| MO (10 ⁹ /L) | Monocyte Count | 0.7 | 0.22 | 0.20 | 0.19 | 0.09 | 0.57 | 0.49 | 0.07 | 0.02 | 0.10 | 0.03 |
| BA (10 ⁹ /L) | Basophil Count | NA | 0.00 | 0.00 | 0.00 | 0.01 | 0.03 | 0.03 | 0.01 | 0.01 | 0.02 | 0.02 |
| EO (10 ⁹ /L) | Eosinophil Count | 0.2 | 0.03 | 0.02 | 0.02 | 0.01 | 0.09 | 0.09 | 0.02 | 0.01 | 0.07 | 0.05 |

Supplementary Table S5: Clinicopathological characteristics of OSCC patients and Syk expression

| | N (%) |
|---------------------------------|----------------------|
| HNSCC | 32 |
| Age (range, median) | 30.33 - 80.27(60.25) |
| Sex | |
| Male | 22 (68.75) |
| Female | 10 (31.25) |
| Stage | |
| I | 12 (37.5%) |
| II | 5 (15.63%) |
| III | 2 (6.25%) |
| IV | 12 (37.5%) |
| Unknown | 1 (3.13%) |
| Extra Capsular Invasion | |
| Positive | 6 (18.75%) |
| Negative | 15 (46.88%) |
| Unknown | 11 (34.38%) |
| Perineural Involvement | |
| Positive | 6 (18.75%) |
| Negative | 15 (46.88%) |
| Unknown | 11 (34.38%) |
| Vascular Involvement | |
| Positive | 3 (9.38%) |
| Negative | 18 (56.25%) |
| Unknown | 11 (34.38%) |
| Follow-up Outcome | |
| Positive | 26 (81.25%) |
| Negative | 6 (18.75%) |
| Syk IHC Analysis | |
| Nuclear positivity (≥ 3) | 20 (62.5%) |
| Nuclear negativity (< 3) | 12 (37.5%) |

*No detectable Syk expression was observed in 16 normal oral tissues analyzed

Supplementary Table S6: Clinicopathological characteristics of OSCC patients and PLK1 expression

| | N (%) |
|-----------------------------------|-------------|
| HNSCC | 30 |
| Age (range, median) | 39-85 (59) |
| Sex | |
| Male | 16 (53.33) |
| Female | 14 (46.67) |
| Stage | |
| I | 5 (16.67%) |
| II | 5 (16.67%) |
| III | 9 (30.0%) |
| IV | 8 (26.67%) |
| Unknown | 3 (10.0%) |
| Extra Capsular Invasion | |
| Positive | 1 (3.33%) |
| Negative | 29 (96.67%) |
| Perineural Involvement | |
| Positive | 6 (20.0%) |
| Negative | 24 (80.0%) |
| Vascular Involvement | |
| Positive | 5 (16.67%) |
| Negative | 25 (83.33%) |
| Follow-up Outcome | |
| Positive | 6 (20.0%) |
| Negative | 24 (80.0%) |
| PLK1 IHC Analysis | |
| Nuclear positivity (≥ 3.5) | 10 (33.33%) |
| Nuclear negativity (< 3.5) | 20 (66.67%) |

*No detectable PLK1 expression was observed in 16 normal oral tissues analyzed

Supplementary Table S7: Multivariate Cox regression analysis for Syk expression in OSCC patients

| OSCC | Kaplan-Meier survival analysis unadjusted p-value | Multivariate Cox regression analysis adjusted p-value | Hazard's ratio (H.R.) | 95% C.I. |
|---------------------------------|---|---|-----------------------|---------------|
| Syk _{Nuc} ⁺ | 0.017 | 0.011 | 0.303 | 0.120 – 0.761 |
| Age | 0.206 | 0.939 | ---- | ---- |
| T Classification | 0.385 | 0.374 | ---- | ---- |
| Nodal Classification | 0.068 | 0.165 | ---- | ---- |
| Clinical Stage | 0.135 | 0.109 | ---- | ---- |

Supplementary Table S8: Multivariate Cox regression analysis for PLK1 expression in OSCC patients

| OSCC | Kaplan-Meier survival analysis unadjusted p-value | Multivariate Cox regression analysis adjusted p-value | Hazard's ratio (H.R.) | 95% C.I. |
|----------------------------------|---|---|-----------------------|---------------------|
| PLK1 _{Nuc} ⁺ | 0.004 | 0.029 | 11.009 | 1.280-94.647 |
| Age | 0.482 | 0.143 | ---- | ---- |
| T Classification | 0.714 | 0.223 | ---- | ---- |
| Nodal Classification | 0.514 | 0.056 | ---- | ---- |
| Clinical Stage | 0.319 | 0.514 | ---- | ---- |

Supplementary Table S9: List of Primers for real time PCR and siRNAs used in this study

| No. | Primer | Sequence |
|-----|-----------------------------|-------------------------------|
| 1 | PLK1 forward | 5'-AGTCGACCACCTCACCTGTC-3' |
| | PLK1 reverse | 5'-GCCCCTCACAGTCCTCAATA-3' |
| 2 | SYK forward | 5'-GATGCTGGTTATGGAGATG-3' |
| | SYK reverse | 5'-TCTATGATGTTCTTATCCTTGAC-3' |
| 3 | PLK4 forward | 5'-AATCAAGCACTCTCCAATC-3' |
| | PLK4 reverse | 5'-TGTGTCCTTCTGCAAATC-3' |
| 4 | GAPDH forward | 5'-CAGAGCAAGAGAGGCATCCT-3' |
| | GAPDH forward | 5'-TTGAAGGTCTCAAACATGAT-3' |
| 5 | siPLK1-1 | 5'-GCACAUACCGCCUGAGUCUtt-3' |
| | | 3'-ttCGUGUAUGGCGGACUCAGA-5' |
| 6 | siPLK1-2 | 5'-CCACCAAGGUUUUCGAUUGtt-3' |
| | | 3'-ttGGUGGUUCCAAAAGCUAAC-5' |
| 7 | siRNA negative control (NC) | 5'-UUCUCCGAACGUGUCACGUtt-3' |
| | | 3'-ttAAGAGGCUUGCACAGUGCA-5' |

Supplementary Table S10: List of antibodies used in this study

| S. No. | Antibody | Catalog No. | Company | Dilution |
|--------|-------------------|-------------|----------------|----------|
| 1 | Syk | sc-1240 | Santa Cruze | 1:1000 |
| 2 | PLK1 | 05-844 | Millipore | 1:1000 |
| 3 | EGFR | ab93051 | Abcam | 1:500 |
| 4 | PARP | 9542 | Cell Signaling | 1:1000 |
| 5 | Caspase 9 | 9502 | Cell Signaling | 1:1000 |
| 6 | Cleaved Caspase 9 | 9505 | Cell Signaling | 1:500 |
| 7 | Caspase 3 | 9962P | Cell Signaling | 1:1000 |
| 8 | Cleaved Caspase 3 | 9664P | Cell Signaling | 1:500 |
| 9 | Akt | C67E7 | Cell Signaling | 1:2000 |
| 10 | p-Akt 308 | C31E5E | Cell Signaling | 1:500 |
| 11 | p-Akt 473 | D9E | Cell Signaling | 1:1000 |
| 12 | Bad | 9292 | Cell Signaling | 1:500 |
| 13 | pBad | S1127E11 | Cell Signaling | 1:1000 |
| 14 | pmTOR | 2974P | Cell Signaling | 1:500 |
| 15 | mTOR | 2983P | Cell Signaling | 1:1000 |
| 16 | pS6 | 4857S | Cell Signaling | 1:1000 |
| 17 | Cyclin D1 | ab134175 | Abcam | 1:1000 |
| 18 | GAPDH | sc-365062 | Santa Cruz | 1:5000 |
| 19 | β - actin | sc-47778 | Santa Cruz | 1:5000 |