**Supplementary Table 1.** Mapping of Hox genes on the current *An. gambiae* genome assembly (AgamP3).

| An. gambiae protein (Vector Base ID) | BLAST identified *Drosophila* or insect homologue* | Actual position on 2R chromosome (NT_078266.2, dated 28-SEP-2011) | Strand |
|-------------------------------------|--------------------------------------------------|---------------------------------------------------------------|--------|
| 1 AGAP004649 labial                 | 59467177..59507503                              | Direct                                                        |        |
| 2 AGAP004648 pb                     | 59327241..59362060                              | Direct                                                        |        |
| 3 AGAP004647 Zen                    | 59304592..59305887                              | Direct                                                        |        |
| 4 AGAP004646 Dfd                    | 59201123..59235934                              | Direct                                                        |        |
| 5 AGAP004659 Scr                    | 59732419..59775031                              | Complement                                                    |        |
| 6 AGAP013157 ftz                    | 59790806..59792241                              | Direct                                                        |        |
| 7 AGAP004660 Antp                   | 59831157..59921675                              | Complement                                                    |        |
| 8 AGAP004661 Ubx                    | 60116706..60166422                              | Complement                                                    |        |
| 9 AGAP004662 abd-A                  | 60236329..60253235                              | Complement                                                    |        |
| 10 AGAP004664 Abd-B                 | 60411039..60422266                              | Complement                                                    |        |

*Protein sequences of *An. gambiae* were used as query to search against the NCBI non-redundant database. The homologue hit was chosen based on the blast similarity. Each of the *An. gambiae* protein gave high scoring hits that restricted to one hox homologue of *Drosophila* or other insects under the default search parameters.*
**Supplementary Table 2.** List of predicted boundary elements from the Hox complex of *An.gmabiae*. The size and the number of motifs for boundary interacting factors, GAF, dCTCF, BEAF, Zw5 and Su(Hw) for each candidate boundary is given. Note that in this study, boundary number 27, 41, 44 and 51 are referred to as *AgB18, AgB1, AgB2* and *AgB7*, respectively.

| Boundary ID | CTCF | GAF | Su(Hw) | Elba | F7M | BEAF | Zw5 | Size (bp) |
|------------|------|-----|--------|------|-----|------|-----|-----------|
| 1          | 0    | 2   | 0      | 0    | 0   | 10   | 1   | 1272      |
| 2          | 0    | 5   | 0      | 1    | 0   | 4    | 2   | 863       |
| 3          | 0    | 1   | 0      | 0    | 0   | 7    | 1   | 750       |
| 4          | 0    | 3   | 0      | 1    | 0   | 1    | 7   | 1169      |
| 5          | 0    | 4   | 0      | 0    | 0   | 7    | 2   | 1223      |
| 6          | 0    | 7   | 0      | 0    | 0   | 3    | 3   | 1311      |
| 7          | 0    | 6   | 0      | 1    | 1   | 12   | 2   | 1988      |
| 8          | 1    | 0   | 0      | 0    | 0   | 2    | 1   | 994       |
| 9          | 0    | 12  | 0      | 0    | 0   | 1    | 2   | 1204      |
| 10         | 0    | 4   | 0      | 0    | 0   | 7    | 2   | 1190      |
| 11         | 2    | 1   | 0      | 0    | 0   | 6    | 0   | 996       |
| 12         | 0    | 7   | 0      | 0    | 0   | 2    | 2   | 926       |
| 13         | 0    | 12  | 0      | 0    | 0   | 6    | 1   | 1634      |
| 14         | 1    | 3   | 0      | 0    | 0   | 1    | 1   | 672       |
| 15         | 1    | 2   | 0      | 0    | 0   | 5    | 0   | 1620      |
| 16         | 1    | 1   | 0      | 0    | 0   | 4    | 2   | 597       |
| 17         | 0    | 6   | 0      | 0    | 0   | 8    | 0   | 1013      |
| 18         | 1    | 2   | 0      | 0    | 0   | 3    | 2   | 1164      |
| 19         | 1    | 3   | 0      | 0    | 0   | 4    | 0   | 1644      |
| 20         | 0    | 7   | 0      | 0    | 0   | 6    | 3   | 1450      |
| 21         | 0    | 12  | 0      | 0    | 0   | 8    | 4   | 1831      |
| 22         | 0    | 2   | 0      | 0    | 0   | 4    | 4   | 875       |
| 23         | 0    | 5   | 0      | 1    | 1   | 1    | 0   | 658       |
| 24         | 1    | 2   | 0      | 0    | 0   | 2    | 3   | 684       |
| 25         | 0    | 1   | 0      | 0    | 0   | 7    | 3   | 755       |
| 26         | 0    | 1   | 0      | 0    | 0   | 7    | 3   | 1033      |
| 27/AgB18   | 0    | 5   | 0      | 0    | 0   | 7    | 2   | 1391      |
| 28         | 0    | 7   | 0      | 1    | 0   | 0    | 3   | 888       |
| 29         | 0    | 3   | 0      | 1    | 0   | 3    | 1   | 699       |
| 30         | 0    | 1   | 0      | 0    | 0   | 6    | 0   | 204       |
| 31         | 0    | 7   | 0      | 0    | 0   | 4    | 2   | 1129      |
| 32         | 0    | 3   | 0      | 1    | 0   | 3    | 2   | 445       |
| 33         | 0    | 7   | 0      | 0    | 0   | 2    | 5   | 1095      |
|    | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|----|---|---|---|---|---|---|---|---|---|---|-----|---|----|----|
| 34 | 1 | 1 | 0 | 0 | 0 | 1 | 1 |   |   |   |     |    |    | 310|
| 35 | 1 | 2 | 0 | 0 | 0 | 6 | 0 |   |   |   |     |    |    | 991|
| 36 | 0 | 2 | 0 | 0 | 0 | 6 | 1 |   |   |   |     |    |    | 731|
| 37 | 0 | 8 | 0 | 1 | 0 | 5 | 3 |   |   |   |     |    |    | 1599|
| 38 | 0 | 3 | 0 | 0 | 0 | 11| 1 |   |   |   |     |    |    | 1441|
| 39 | 0 | 7 | 0 | 0 | 0 | 11| 3 |   |   |   |     |    |    | 2002|
| 40 | 0 | 0 | 0 | 0 | 0 | 7 | 1 |   |   |   |     |    |    | 698 |
| 41/AgB1 | 0 | 12| 0 | 1 | 0 | 1 | 2 |   |   |   |     |    |    | 1489|
| 42 | 1 | 0 | 0 | 0 | 0 | 2 | 0 |   |   |   |     |    |    | 84  |
| 43 | 0 | 0 | 0 | 0 | 0 | 5 | 3 |   |   |   |     |    |    | 669 |
| 44/AgB2 | 0 | 7 | 0 | 0 | 0 | 5 | 2 |   |   |   |     |    |    | 1233|
| 45 | 0 | 1 | 1 | 0 | 0 | 4 | 2 |   |   |   |     |    |    | 709 |
| 46 | 0 | 1 | 0 | 0 | 0 | 11| 0 |   |   |   |     |    |    | 1272|
| 47 | 0 | 3 | 0 | 0 | 0 | 6 | 1 |   |   |   |     |    |    | 863 |
| 48 | 0 | 3 | 0 | 0 | 0 | 6 | 1 |   |   |   |     |    |    | 863 |
| 49 | 0 | 16| 0 | 0 | 0 | 2 | 0 |   |   |   |     |    |    | 577 |
| 50 | 0 | 6 | 0 | 0 | 0 | 4 | 0 |   |   |   |     |    |    | 860 |
| 51/AgB7 | 0 | 24| 1 | 1 | 0 | 1 | 0 |   |   |   |     |    |    | 869 |
| 52 | 0 | 10| 0 | 0 | 0 | 4 | 0 |   |   |   |     |    |    | 1177|
| 53 | 0 | 3 | 0 | 0 | 0 | 6 | 0 |   |   |   |     |    |    | 786 |
| 54 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |   |   |   |     |    |    | 159 |
**Supplementary Table 3.** List of predicted enhancers in mosquito Hox complex.

| Enhancer ID | Start   | Stop    | Size (bp) | Motifs       |
|-------------|---------|---------|-----------|--------------|
| 1           | 38114   | 38187   | 74        | Kr-Hb-Hb     |
| 2           | 45543   | 45665   | 123       | Hb-Hb-Kr-Hb  |
| 3           | 54354   | 54434   | 81        | Hb-Hb-Kr     |
| 4           | 58589   | 58783   | 195       | Hb-Hb-Kr     |
| 5           | 93352   | 93646   | 295       | Hb-Kr-Hb     |
| 6           | 116200  | 116240  | 41        | Hb-Hb-Kr     |
| 7           | 161126  | 161341  | 216       | Hb-Kr-Hb     |
| 8           | 167098  | 167429  | 332       | Hb-Hb-Hb-Kr-Hb |
| 9           | 176934  | 177136  | 203       | Kr-Kr-Hb     |
| 10          | 185605  | 185999  | 395       | Hb-Kr-Hb-Hb-Kr-Hb |
| 11          | 193201  | 193356  | 156       | Kr-Hb-Hb     |
| 12          | 242194  | 242348  | 155       | Hb-Kr-Kr     |
| 13          | 245890  | 245988  | 99        | Hb-Hb-Kr     |
| 14          | 268918  | 269095  | 178       | Kr-Hb-Hb     |
| 15          | 275064  | 275309  | 246       | Hb-Kr-Hb-Kr  |
| 16          | 281332  | 281627  | 296       | Hb-Kr-Hb     |
| 17          | 290143  | 290286  | 144       | Kr-Hb-Hb     |
| 18          | 311261  | 311412  | 152       | Hb-Hb-Kr     |
| 19          | 372846  | 373090  | 245       | Hb-Kr-Hb-Hb  |
| 20          | 535076  | 535370  | 295       | Hb-Kr-Hb     |
| 21          | 536477  | 536715  | 239       | Hb-Hb-Kr     |
| 22          | 542729  | 542916  | 188       | Hb-Kr-Hb     |
| 23          | 565350  | 565678  | 329       | Hb-Kr-Hb-Hb-Kr-Hb |
| 24          | 567190  | 567332  | 143       | Hb-Kr-Hb     |
| 25          | 609998  | 610275  | 278       | Hb-Hb-Kr     |
| 26          | 645032  | 645253  | 222       | Hb-Kr-Hb-Kr  |
| 27          | 669557  | 669804  | 248       | Hb-Kr-Hb     |
| 28          | 701975  | 702364  | 390       | Hb-Hb-Kr-Hb  |
| 29          | 710844  | 710945  | 102       | Kr-Hb-Hb     |
| 30          | 714262  | 714411  | 150       | Kr-Hb-Hb     |
| 31          | 754342  | 754602  | 261       | Kr-Hb-Hb     |
| 32          | 786767  | 786963  | 197       | Hb-Kr-Hb     |
| 33          | 797491  | 797792  | 302       | Hb-Hb-Kr-Hb  |
| 34          | 803447  | 803604  | 158       | Hb-Kr-Hb     |
| 35          | 913078  | 913292  | 215       | Hb-Hb-Kr     |
| 36          | 923399  | 923671  | 273       | Hb-Kr-Hb-Hb  |
| 37          | 934029  | 934214  | 186       | Hb-Kr-Hb     |
| 38          | 956064  | 956513  | 450       | Hb-Kr-Hb-Hb  |
| 39          | 1060781 | 1061067 | 287       | Hb-Hb-Kr     |
| 40          | 1082884 | 1083059 | 176       | Kr-Hb-Hb     |
| 41          | 1095300 | 1095473 | 174       | Hb-Kr-Hb     |
| 42          | 1126475 | 1126625 | 151       | Kr-Hb-Hb     |
| 43          | 1126956 | 1127287 | 332       | Hb-Kr-Hb-Kr  |
| 44          | 1131395 | 1131677 | 283       | Hb-Hb-Hb-Hb-Kr-Hb |
| 45          | 1136499 | 1136765 | 267       | Hb-Kr-Kr     |
| 46          | 1146447 | 1146724 | 278       | Kr-Hb-Kr     |
| 47          | 1150414 | 1150699 | 286       | Hb-Hb-Kr     |
| 48          | 1160912 | 1161221 | 310       | Hb-Hb-Kr-Hb  |
| 49          | 1205562 | 1205850 | 289       | Kr-Hb-Hb     |
| 50          | 1209677 | 1209919 | 243       | Kr-Hb-Hb     |
| 51          | 1216891 | 1217102 | 212       | Hb-Kr-Hb-Hb  |
**Supplementary Table 4.** List of oligos used in the study.

| Oligo Name | Sequence (5’ to 3’) |
|------------|---------------------|
| AgHox-B1- F | TATAGGGCCCCGCATAGGTTCTCTCGACT |
| AgHox-B1- R | TATAGGGCCCATATCGTGGCAAGTTTGCTAT |
| AgHox-B2-F | TATAGGGCCAGGGGTAGGAGTTTCGTT |
| AgHox-B2-R | TATAGGGCCCGAATTATTTGTCTCTTACACCC |
| AgHox-B7-F | TATAGGGCCTGTGTGGTTAATGTATCTGCAAG |
| AgHox-B7-R | TATAGGGCCCAAAACTTGGTTAATTGATGAAA |
| AgHox-B18-F | TATAGGGCCCGGATTAACGATAATTGTTTG |
| B1-ChIP2-F | TGGAGCAAAGTCTTTTGCAG |
| B1-ChIP2-R | AAGGTAACCGGTATCAACAGTAGC |
| B2-ChIP2-F | GACGAAGTCTAACAAGAGACACA |
| B2-ChIP2-R | CTGTTGTCTCCATCGCAACC |
| B7-ChIP1-F | GGGCCCTAGAATAATGTGACGG |
| B7-ChIP1-R | CTCTCCTCTCTCTTTTCGTCTGATC |
| B18-ChIP2-F | GAGCTCTACTAAACAAATCAGGG |
| B18-ChIP2-R | GCTTACACTCCACGGTTAATC |
| MW3-F | ACTGCGATGGCAACATCAAA |
| MW3-R | TAGCGAGCACTACCAGA |
| iab7-PRE-IF | GGAATACCGCTCGTGTAGG |
| iab7-PRE-IR | GCAGCCATCATGGATGTAAG |